

Supporting Information

Dutton *et al.* 10.1073/pnas.0804621105

SI Methods

We analyzed the protein sequences of these genomes with Phobius, <http://phobius.sbc.su.se> (1) and a Prosite profile for lipoproteins, release 20.0, www.expasy.ch/prosite (2). Phobius is a subcellular localization prediction program based on SignalP 3.0 and TMHMM 2.0. Thus, proteins exported by the general secretion machinery, SecYEG, should be detected, as well as many proteins that are exported by the major alternative pathway for export in many bacteria, the TAT pathway (3), which utilizes signal sequences that are very similar to Sec pathway signal sequences. The Sec system is universally conserved and signals in secreted and transmembrane proteins that determine secretion and topology are similar across all bacteria. Such signals, as identified by the methods we used, are variable but always a significant fraction of all of the proteins in each bacterial genome. Thus we believe our approach is adequate for estimation of gross statistical features of the distribution of cysteine residues in exported proteins of most, if not all organisms.

For each protein, each amino acid residue was assigned to one of six classes, based on its predicted subcellular localization. Thus, each amino acid within a protein was assigned to one of the following classes: cytoplasmic (class 1); transmembrane protein-cytoplasmic domains (class 2); transmembrane protein-inner membrane spanning helices (class 3); transmembrane protein-periplasmic domains (class 4); exported protein, directed by a signal sequence whether the final destination is the periplasm, the outer membrane or outside of the cell (class 5); and other, which includes residues predicted to be in cleavable signal sequences and the amino terminal cysteine residues of mature lipoproteins (class 6). Transmembrane proteins with predicted signal sequences were classified as transmembrane.

For each genome, we calculated two numbers for each of the twenty amino acids in each class. The first is the even fraction, the fraction of proteins with even numbers of that amino acid of that class, excluding proteins with none of that amino acid in that class. We term that number the even fraction, or Efrac. The second number, the AApref, is a measure of the preference for

or bias against that amino acid in that class. This is calculated from the amino acid composition of the class and the amino acid composition of the whole proteome. It is the ratio of the frequency of the amino acid in the class to the frequency in the genome. This is the same as the ratio of the fraction of the amino acid that is in the class to the fraction of all amino acids that is in the class.

To assess the significance of the Efrac, we carried out a randomization procedure to obtain a mean value and standard deviation for the Efrac of each amino acid expected at random. We used two different randomization procedures for *E. coli*. In the first, we simply randomized the sequences of each class, keeping the overall amino acid composition of the class constant. By repeating this procedure 1,000 times and averaging the even fractions, we obtained mean and standard deviation values. Repetition of the entire process produced identical or nearly identical results. In the second method, we used random numbers generated according to the Poisson distribution to get counts for each protein. The Poisson parameter, lambda, was set to the number of amino acids of that protein in that class times the frequency of the amino acid in the class. Again repetition for 1000 times and averaging gave a mean and standard deviation which was used to calculate a z score, the number of standard deviations between the random mean and the observed value of the Efrac. We used a Perl interface to the C library, RANDLIB, obtained from Comprehensive Perl Archive Network, www.cpan.org. This method gave the same result as that described above for *E. coli* and was used to all other genomes since it is computationally faster.

DsbA homologs with a cytoplasmic localization, based on Phobius predictions, were excluded. Since the Pfam DsbB HMM model missed some known DsbB homologs found in the alpha-proteobacteria, we built an additional DsbB HMM model (based on alpha-proteobacterial DsbB sequences) to supplement the homology searches. We also used BLASTP (4) to identify additional DsbA homologs using the *Staphylococcus aureus* DsbA (gi11935158) as a query and collected hits below the e value of $<10^{-4}$.

1. Kall L, Krogh A, Sonnhammer EL (2007) Advantages of combined transmembrane topology and signal peptide prediction—The Phobius web server. *Nucleic Acids Res* 35:W429–W432.
2. Hulo N, *et al.* (2006) The PROSITE database. *Nucleic Acids Res* 34:D227–D230.

3. Lee PA, Tullman-Ercek D, Georgiou G (2006) The bacterial twin-arginine translocation pathway. *Annu Rev Microbiol* 60:373–395.
4. Altschul SF, *et al.* (1990) Basic local alignment search tool. *J Mol Biol* 215:403–410.

	<i>Burkholderia cepacia</i> AMMD	+	+	-	18.075	0.65	0.7	0.4 +/- 0.017		
	<i>Burkholderia mallei</i> ATCC 23344	+	+	-	7.761	0.65	0.593	0.416 +/- 0.023		Host-associated
	<i>Burkholderia pseudomallei</i> 1710b	+	+	-	10.732	0.68	0.63	0.422 +/- 0.019	Aerobic	Terrestrial
	<i>Burkholderia pseudomallei</i> K96243	+	+	-	14.294	0.65	0.672	0.407 +/- 0.019	Aerobic	Terrestrial
	<i>Burkholderia thailandensis</i> E264	+	+	-	12.112	0.68	0.644	0.414 +/- 0.019	Aerobic	Terrestrial
	<i>Burkholderia xenovorans</i> LB400	+	+	-	13.454	0.62	0.617	0.398 +/- 0.016	Aerobic	Multiple
	<i>Chromobacterium violaceum</i>	+	+	-	12.904	0.72	0.691	0.417 +/- 0.021	Facultative	Multiple
	<i>Dechloromonas aromatica</i> RCB	+	+	-	14.769	0.8	0.721	0.417 +/- 0.021	Facultative	Multiple
	<i>Methylobacillus flagellatus</i> KT	+	+	-	9.824	0.61	0.644	0.373 +/- 0.028	Aerobic	Specialized
	<i>Neisseria gonorrhoeae</i> FA 1090	+	+	-	6.522	0.55	0.609	0.38 +/- 0.035	Aerobic	Host-associated
	<i>Neisseria meningitidis</i> MCS8	+	+	-	8.221	0.53	0.669	0.376 +/- 0.036	Aerobic	Host-associated
	<i>Neisseria meningitidis</i> Z2491	+	+	-	7.679	0.56	0.66	0.38 +/- 0.036	Aerobic	Host-associated
	<i>Nitrosomonas europaea</i>	+	+	-	10.536	0.58	0.721	0.394 +/- 0.031	Aerobic	Multiple
	<i>Nitrosospira multiformis</i> ATCC 25196	+	+	-	8.971	0.69	0.656	0.397 +/- 0.029	Aerobic	Terrestrial
	<i>Polaromonas JS666</i>	+	+	-	13.763	0.59	0.67	0.393 +/- 0.02	Aerobic	Multiple
	<i>Ralstonia eutropha</i> H16	+	+	-	14.291	0.57	0.65	0.391 +/- 0.018	Facultative	Specialized
	<i>Ralstonia eutropha</i> JMP134	+	+	-	16.372	0.57	0.681	0.389 +/- 0.018	Facultative	Multiple
	<i>Ralstonia metallidurans</i> CH34	+	+	-	14.902	0.61	0.656	0.397 +/- 0.017	Facultative	Specialized
	<i>Ralstonia solanacearum</i>	+	+	-	13.108	0.69	0.664	0.403 +/- 0.02	Aerobic	Multiple
	<i>Rhodoferrax ferrireducens</i> T118	+	+	-	10.322	0.77	0.649	0.427 +/- 0.022		
	<i>Thiobacillus denitrificans</i> ATCC 25259	+	-	+	9.453	0.72	0.642	0.392 +/- 0.026	Facultative	Multiple
Alpha	<i>Agrobacterium tumefaciens</i> C58_UWash	+	+	-	12.223	0.77	0.67	0.407 +/- 0.022	Aerobic	Multiple
	<i>Anaplasma marginale</i> St. Maries	+	+	-	0.105	0.86	0.479	0.474 +/- 0.049	Aerobic	Host-associated
	<i>Anaplasma phagocytophilum</i> HZ	+	+	-	1.431	0.72	0.533	0.461 +/- 0.05	Aerobic	Host-associated
	<i>Bartonella henselae</i> Houston-1	+	+	-	6.42	0.62	0.677	0.403 +/- 0.043	Aerobic	Host-associated
	<i>Bartonella quintana</i> Toulouse	+	+	-	4.848	0.58	0.628	0.4 +/- 0.047	Aerobic	Host-associated
	<i>Bradyrhizobium japonicum</i>	+	+	-	15.061	0.81	0.66	0.418 +/- 0.016	Aerobic	Host-associated
	<i>Brucella abortus</i> 9-941	+	+	-	9.148	0.79	0.67	0.399 +/- 0.03	Facultative	Host-associated
	<i>Brucella melitensis</i>	+	+	-	7.786	0.85	0.653	0.409 +/- 0.031	Aerobic	Host-associated
	<i>Brucella melitensis</i> biovar Abortus	+	+	-	9.767	0.79	0.687	0.401 +/- 0.029		
	<i>Brucella suis</i> 1330	+	+	-	8.745	0.82	0.642	0.405 +/- 0.027	Aerobic	Host-associated
	<i>Candidatus Pelagibacter ubique</i> HTCC1062		+	-	7.022	0.78	0.747	0.408 +/- 0.048	Aerobic	Aquatic
	<i>Caulobacter crescentus</i>	+	+	-	12.223	0.73	0.677	0.405 +/- 0.022	Aerobic	Aquatic
	<i>Ehrlichia canis</i> Jake	+	+	-	2.046	0.9	0.58	0.48 +/- 0.049		Host-associated
	<i>Ehrlichia chaffeensis</i> Arkansas	+	+	-	1.069	0.77	0.519	0.461 +/- 0.054		Host-associated
	<i>Ehrlichia ruminantium</i> Gardel	+	+	-	2.409	0.74	0.603	0.464 +/- 0.058		Host-associated
	<i>Ehrlichia ruminantium</i> str. Welgevonden	+	+	-	2.381	0.71	0.587	0.455 +/- 0.055		Host-associated
	<i>Ehrlichia ruminantium</i> Welgevonden	+	+	-	2.916	0.72	0.635	0.467 +/- 0.058		Host-associated
	<i>Erythrobacter litoralis</i> HTCC2594	+	+	-	15.011	0.8	0.791	0.406 +/- 0.026	Aerobic	Aquatic
	<i>Gluconobacter oxydans</i> 621H	+	+	-	8.884	0.6	0.674	0.41 +/- 0.03	Aerobic	Multiple
	<i>Granulobacter bethesdensis</i> CGDNIH1	+	+	-	7.794	0.76	0.653	0.41 +/- 0.031		Multiple
	<i>Hyphomonas neptunium</i> ATCC 15444	+	+	-	16.445	0.74	0.764	0.404 +/- 0.022	Aerobic	Aquatic
	<i>Jannaschia</i> CCS1	+	+	-	16.889	0.84	0.797	0.411 +/- 0.023	Aerobic	Aquatic
	<i>Magnetospirillum magneticum</i> AMB-1	+	+	-	11.93	0.83	0.686	0.421 +/- 0.022	Microaerophilic	Aquatic
	<i>Maricaulis maris</i> MCS10	+	+	-	18.313	0.66	0.809	0.394 +/- 0.023	Facultative	Aquatic
	<i>Mesorhizobium</i> BNC1	+	+	-	13.866	0.83	0.711	0.411 +/- 0.022	Aerobic	Multiple
	<i>Mesorhizobium loti</i>	+	+	-	15.067	0.92	0.704	0.417 +/- 0.019	Aerobic	Multiple
	<i>Neorickettsia sennetsu</i> Miyayama	+	+	-	0.851	0.83	0.507	0.454 +/- 0.063		Multiple
	<i>Nitrobacter hamburgensis</i> X14	+	+	-	13.268	0.89	0.7	0.405 +/- 0.022	Aerobic	Terrestrial
	<i>Nitrobacter winogradskyi</i> Nb-255	+	+	-	9.139	0.84	0.652	0.409 +/- 0.027	Facultative	Terrestrial

Table S1 cont.

	Methanosarcina barkeri fusaro	-	-	-	1.099	0.83	0.495	0.461 +/- 0.031		
	Methanosarcina mazei	-	-	-	0.443	0.77	0.465	0.446 +/- 0.043	Anaerobic	Multiple
	Methanospirillum hungatei JF-1	-	-	-	1.451	0.64	0.49	0.445 +/- 0.031	Anaerobic	Multiple
Methanopyri	Methanopyrus kandleri	-	-	-	0.325	0.98	0.49	0.473 +/- 0.052	Anaerobic	Specialized
Thermococci	Pyrococcus abyssi	-	-	-	-0.06	0.99	0.398	0.402 +/- 0.063	Anaerobic	Aquatic
	Pyrococcus furiosus	-	-	-	0.929	0.88	0.438	0.392 +/- 0.05	Anaerobic	Aquatic
	Pyrococcus horikoshii	-	-	-	2.283	1.03	0.485	0.377 +/- 0.047	Anaerobic	Aquatic
	Thermococcus kodakaraensis KOD1	-	-	-	2.086	1	0.495	0.4 +/- 0.045	Anaerobic	Specialized

Table S1 cont.