Supporting Information for

B. subtilis Ribosomal Proteins: Structural Homology and Post-translational Modifications

*Matthew A. Lauber, William E. Running and James P. Reilly**

Department of Chemistry, Indiana University, Bloomington, Indiana 47405

*Author for correspondence: James P. Reilly
E-mail: reilly@indiana.edu

Supplemental Table 1. SCX Dimension Gradient. Mobile phase A: 6 M urea, 20 mM acetic acid, pH 5.1. Mobile phase B: 6 M urea, 500 mM NaCl, 20 mM acetic acid, pH 5.1.

Time	Flow rate (µL/min)	%A	%B
0	150	100	0
20	300	100	0
25	300	100	0
45	300	90	10
95	300	65	35
96	300	30	70
102	300	0	100
103	300	100	0
110	300	100	0

Supplemental Table 2. RPLC Gradient. Mobile Phase A: 0.2% formic acid, 0.1% trifluoroacetic acid in water. Mobile phase B: 0.2% formic acid, 0.1% trifluoroacetic acid in acetonitrile.

Time	%A	%B
0.0	100	0
7.0	100	0
7.1	80	20
37.0	30	70
40.0	10	90
44.0	10	90
44.1	100	0
53.0	100	0

Supplemental Table 3. Peptide MS/MS matches from which sequencing errors were confirmed. Corrected sequencing errors are shown in red.

protein	sequencing error	observed m/z	M_r(expt)	M_r(calc)	mass error	Mascot ion score[#]	peptide
L1	H17R	875.4809	2623.4209	2623.4221	-0.0012	100	K.YVEAAKLVDRSKAYDVSEAVALVK.K
	S53R	513.6179	1537.8318	1537.8325	-0.0007	54	R.LGVDPRKNDQQIR.G
	H56D	760.7564	2279.2473	2279.2458	0.0015	76	R.KNDQQIRGAVVLPNGTGKTQR.V
L10	S52F	915.4644	1828.9142	1828.9141	0.0001	70	R.EANVEFKVYKNTMTR.R
	P131S	864.4815	1726.9484	1726.9465	0.0019	114	K.VSTVEEVKALAELPSR.E
	K144Q	863.5004	1724.9862	1724.9859	0.0004	68	R.EGLLSMLLSVLQAPVR.N
L11	V35I	905.2449	3616.9506	3616.9374	0.0132	n/a	K.LQIPAGK*ANPAPPVGPALGQAGVNIMGFCK*EFNAR.T
L13	C16W	757.4466	1512.8787	1512.8776	0.0010	114	R.KWLVVDAAGKTLGR.L
L23	E254K	1024.5035	2046.9924	2046.9932	-0.0007	85	R.SADLM^{ox}TEKKYTFEVDVR.A
	A39V	917.9953	1833.9760	1833.9836	-0.0076	136	R.ANKTEVKDAVESIFGVK.V
L33.1	R13G	703.8223	1405.6300	1405.6324	-0.0024	31	R.VNITLAC*TEC*GER.N
S7	P39S	921.0125	1840.0104	1840.0094	0.0010	112	K.SQTILYKSFDIIKER.T
S12	P128S	518.8111	1035.6075	1035.6077	-0.0001	27	R.SKYGTKKPK.A

[#] Scores greater than 25 are significant ($p<0.05$)

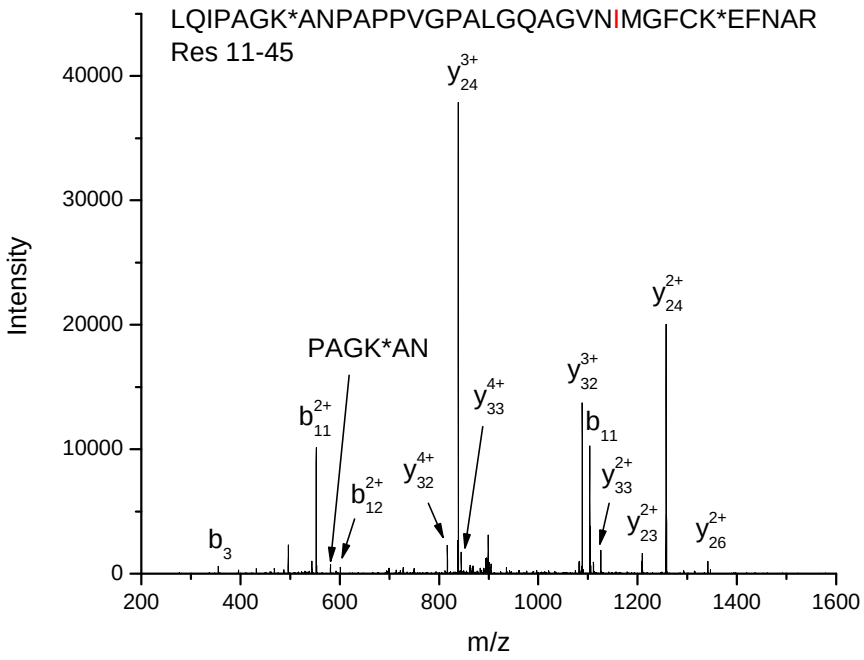
* L11, trimethylated lysine residue
L33.1, intrapeptide disulfide

n/a 3+ and 4+ charge state fragment ions were not matched by Mascot
A manually interpreted MS/MS spectrum is provided in Supplemental Figure 1

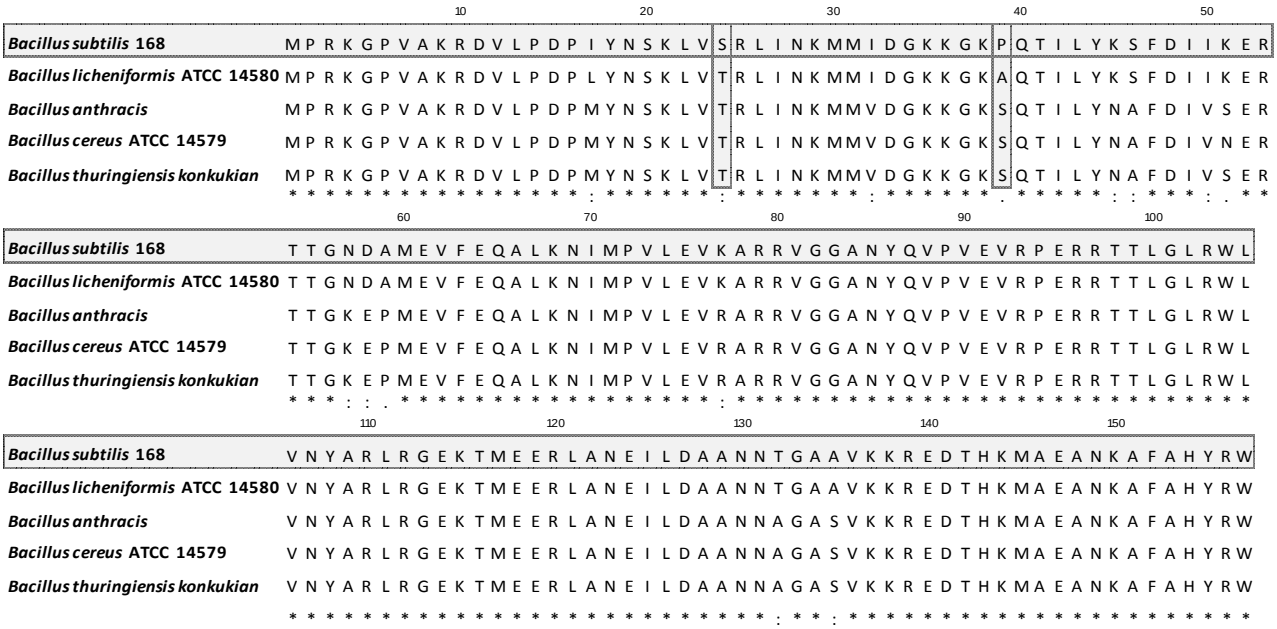
Supplemental Table 4. Peptide MS/MS matches from which conflicts between the published reference genome and other sequencing information have been resolved. Corrected sequencing errors are shown in red.

protein	sequencing error	observed m/z	M_r(expt)	M_r(calc)	mass error	Mascot ion score[#]	peptide
L2	E256G	830.9469	1659.8792	1659.8807	-0.0015	103	R.KSPMSPWGKPTLGFK.T
L18	Y37N	925.1491	2772.4254	2772.4294	-0.0039	163	R.SNKHIYAQIIDDVNGVTLASASTLDK.D
L19	-MKT	682.3602	1362.7058	1362.7064	-0.0006	87	-.M^{ox}QKLIEDITKE.Q
S3	P164S	835.7628	2504.2666	2504.2659	0.0007	107	R.LGGADIARSEYYSEGTVPLHTLR.A
S8	P37A	486.2978	970.5811	970.5811	0.0000	63	R.EIAEILKR.E
	S63F	684.3572	1366.6998	1366.6993	0.0005	65	R.VFLKYGQNNER.V
S12	N102D +R105G	442.2714	882.5283	882.5287	-0.0004	52	R.VKDLPGVR.Y
S13	T85S	638.8725	1275.7304	1275.7299	0.0005	35	R.LIEIGSYRGIR.H

[#] Scores greater than 25 are significant ($p<0.05$)



Supplemental Figure 1. MS-MS spectrum of an L11 peptide containing the V35I sequencing error. The asterisks denote trimethylated lysine residues. Residue position 35 is indicated in red.



Supplemental Figure 2. Multiple sequence alignment of ribosomal protein S7. Suspected sequencing errors (S24T) and (P39S) are indicated.

Supplemental Table 5. Mapped modified sites of ribosomal proteins amidinated before disassembly of the ribosome.

protein	mod. residues
L1	K19, K32, K37
L2	K68, K101
L3	K189, K196[a], K203[b]
L5	K102, K179
L6	K82
L7/L12	K84, K112, K123
L10	K116, K155
L13	K100
L17	K46
L18	K88, K119
L20	K59[c], K84
L22	K27
L30	K49, K57
L35	K59[b], K66
S2	K109
S3	K53
S4	K42, K43, K100[a], K134
S5	K148
S6	K80, K92
S7	K21
S8	K121
S10	K24, K30
S11	(K15 or K16)
S13	K35, K55
S15	K58, K84
S19	K7, K56

[a] consistent only with T. thermophilus crystal structure
[b] consistent only with E. coli crystal structure
[c] not expected based on either crystal structure