

Supplementary Data for:

The Cpx envelope stress response modifies peptidoglycan cross-linking via the L,D-transpeptidase LdtD and the novel protein YgaU

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Table S1. Peptidoglycan composition of *ygaU* mutants over-expressing *ygaU*.

Muropeptide	Average value, mol % (% MC4100 value) ^a :			Average value, mol % (% <i>cpxA24</i> value) ^a :		
	MC4100 ^b	MC4100 <i>ygaU</i> ^b	MC4100 <i>ygaU</i> (<i>pygaU</i>)	<i>cpxA24</i> ^b	<i>cpxA24 ygaU</i> ^b	<i>cpxA24 ygaU</i> (<i>pygaU</i>)
Monomers	67.92	64.5 (95)	56.04 (64)	63.97	63.38 (99)	59.31 (93)
Dimers	29.3	32.96 (112)	42.06 (144)	32.25	33.24 (103)	38.72 (120)
Trimers	2.76	2.53 (92)	1.9 (69)	3.76	3.37 (90)	1.96 (52)
DAD-DAP	3.86	4.12 (107)	15.91 (412)	5.33	8 (150)	11.45 (215)
Lipo	10.4	10.18 (98)	8.8 (85)	9.86	7.92 (80)	8.24 (84)
Anhydro	4.34	4.03 (93)	7 (161)	4.63	4.47 (97)	8.1 (175)
Penta	0.46	0.56 (122)	2.38 (517)	0.58	0.62 (107)	1.16 (200)
Cross-Linking	34.82	38.03 (109)	45.86 (132)	39.79	39.99 (101)	42.65 (107)
D-D/total	11.11	10.47 (94)	34.69 (13)	13.27	19.19 (145)	26.85 (202)
Length	24.46	25.21 (103)	14.28 (22)	22.44	22.36 (100)	12.34 (55)
D33D	0.65	0.8 (123)	5.91 (909)	1.19	1.65 (139)	4.99 (419)
D43D	3.54	2.23 (63)	5.25 (148)	4.06	6.79 (167)	2.77 (62)
D33DL	0.58	1.18 (203)	1.95 (336)	0.58	1.16 (200)	1.56 (269)
D43DN	0.45	0.76 (169)	0.45 (100)	0.64	0.14 (22)	0.48 (75)
T443D	0.21	0.13 (62)	0.66 (314)	0.44	0.72 (164)	0.94 (214)
M3	9.18	8 (87)	12.34 (134)	8.95	6.9 (77)	13.86 (155)
M4G	1.83	2.13 (116)	4.87 (266)	2.61	3.66 (140)	4.24 (162)
M4	28.58	23.61 (83)	7.87 (28)	23.05	25.27 (110)	9.14 (40)
M2	1.43	2.74 (192)	1.36 (95)	1.5	0.74 (49)	1.08 (72)
M5	0.17	0.22 (129)	0.76 (447)	0.13	0.19 (146)	0.22 (169)
M3L	5.09	3.14 (62)	2.15 (42)	4.34	3.3 (76)	2.05 (47)
M3N	0	0	0.94	0	0	0.98
M4N	0.87	0.26 (30)	0.36 (41)	0.58	0.45 (78)	0.95 (164)

a. The molar ratio of the different muropeptide structures was calculated for the indicated strains as previously described for sacculi derived from MC4100, MC4100 *ygaU*, MC4100 *ygaU* (*pygaU*), *cpxA24*, *cpxA24 ygaU*, and *cpxA24 ygaU* (*pygaU*) strains (16). All the strains were grown at 30°C. The different muropeptide structures are abbreviated according to length; (M) monomer, (D) cross-linked dimer, or (T) cross-linked trimer.

The first ten rows indicate general peptidoglycan structure. The numbers indicate the length of the stem peptide, and the last letter indicates whether the muropeptide contains a DAP-DAP cross-link (D), is anchored to Braun's lipoprotein (L), or if there is a 1,6-anhydro muramic acid residue (N), or a glycine (G) at the end of the stem peptide. D-D/total is the ratio of DAP-DAP crosslinks to the total crosslinking. The average of three measurements from biological replicates is shown. Shaded boxes indicate measurements that were different from the MC4100 value (left side of table) or the *cpxA24* value (right side of table) by 25% or more. Numbers in parentheses indicate the % of the MC4100 value (left side of table) or % of the *cpxA24* value (right side of table).

b. The muropeptide composition for these strains was determined once, and is indicated in Tables 3, 4, 5, and S1.