

Supplementary Tables

Table S1 Bacterial strains used in this study

Strain	Relevant characteristics	Reference of source
<i>Streptococcus pneumoniae</i>		
TIGR4	Prototroph	Purchased from ATCC
ATCC49619	Drug sensitive	Purchased from ATCC
S1	TEL resistance clinical isolate	(10)
Sp224	TIGR4 harboring pTKY1041	This study
Sp274	$\Delta tlrB::aad(9)$ in S1	(10)
Sp303	$\Delta tlrB::aad(9)$ in TIGR4	(10)
Sp322	Sp303 harboring pTKY1111	This study
Sp331	$\Delta SP_1901::aad(9)$ in TIGR4	This study
Sp332	$\Delta rlmCD::aad(9)$ in TIGR4	This study
Sp338	Sp332 harboring pTKY1041	This study
Sp345	$\Delta rlmCD::aad(9)$ in S1	This study
Sp355	Sp345 harboring pTKY1111	This study
Sp360	Sp345 harboring pTKY1196	This study
Sp361	Sp332 harboring pTKY1196	This study
Sp369	$\Delta rlmCD::erm(B)$ in Sp303	This study
Sp370	Sp332 harboring pTKY1121	This study

Table S2 Plasmids used in this study

Plasmid	Relevant characteristics	Reference of source
pUC18	Cloning vector	Lab. collection
pLZ12-Km2	Shuttle vector	(17)
pAM225	Shuttle vector:pBR325 carrying Tn917	(18)
pGEX-6p-1	GST gene fusion vector	GE Healthcare
pTKY862	pLZ12-Km2 with Sp resistant cassette <i>aad(9)</i>	(17)
pTKY1041	pLZ12-Km2 with 1401 bp <i>erm(B)</i> fragment from S1	(10)
pTKY1109	pUC18 with 328 bp <i>tlrB</i> fragment	(10)
pTKY1110	pUC18 with disrupted <i>tlrB</i> fragment by insertion of an Sp resistant cassette <i>aad(9)</i>	(10)
pTKY1111	pLZ12-Km2 with 1065 bp <i>tlrB</i> fragment from S1	(10)
pTKY1121	pLZ12-Km2 with 1065 bp <i>tlrB</i> fragment from TIGR4	This study
pTKY1156	pUC18 with bp <i>SP_1901</i> fragment	This study
pTKY1157	pUC18 with 1240 bp <i>rlmCD</i> fragment	This study
pTKY1161	pUC18 with disrupted <i>SP_1901</i> fragment by insertion of an Sp resistant cassette <i>aad(9)</i>	This study
pTKY1174	pUC18 with disrupted <i>rlmCD</i> fragment by insertion of an Sp resistant cassette <i>aad(9)</i>	This study
pTKY1196	pLZ12-Km2 with 2112 bp <i>rlmCD</i> fragment from S1	This study
pTKY1201	pGEX6p-1 with 864 bp <i>tlrB</i> fragment from TIGR4	This study
pTKY1205	pUC18 with disrupted <i>rlmCD</i> fragment by insertion of an Em resistant cassette <i>erm(B)</i> .	This study

Supplementary Figures

Figure S1 Amino acid alignment of *E. coli* RlmC, *S. pneumoniae* SP_1029 and SP_1901, and *B. subtilis* YefA.

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E.coli RlmC      1 -----MQ 2
Sp1029          1 -----MLKKNDIVEVEIVDLTHEGAGVAKVDGLVFFVENALPSEKILMRVLKVNKKIGFGKVEKYLVSQPHRNQDLDLAY 75
Sp1901          1 -----MGINGEGIGFYQKTLVFPVPGALKGEDIYCQITSIRRNFFVEAKLLKVNKSKFRIVPSCSTIY 61
B.subtilis YefA 1 MKMKPPVEKNEYDVTFEDLTHEGAGVAKVQGFPIFVFNALPEEKAQIKVTRVKKGFAGRLIELKEESPVRTDAPCPYI 80

E.coli RlmC      3 CALYDAGRCSRSCQWIMQPIPEQLSAKTADLKNLLADFPVEEWCAPVSGPEQGFENKAKMVSQSVVEKPLLCMLHRDG-TP 81
Sp1029          76 LRSGIADLGHLSYPEQLKFKTKQVKDSLYKIA--GIADVEVAETLGMHPVKYRNKAQVPVRRVNGVLETGFFRKNSHNL 153
Sp1901          62 NECGGCQIMHLHYDKOLEFKTDLHLQALKKFAPAGYENYERPTIGMQEPKYRRAKLFQTRKFKNQVKAQLYAQNSHYL 141
B.subtilis YefA 81 KQCGGCQLQHMTYEGQLLFKQKQVKDVLERIGKLDLSKVTVHPITLGMEDPWNVYRNKAQVPVGEREGGLVAGFYQQRSHDI 160

E.coli RlmC      82 EDLCDCPLYPASFAPVFAALPKFPIARAGLTPYNVARKGELKYLTLTESQSDGGMMLRFVLRSDTKLAQLRKALPWLHEQ 161
Sp1029          154 MPLEDFFIQDPVIDQVVVALRDLLRRFDLKPYPDEKESGLIRNLVVRRGHYSQQIMVVLV-TTRPKVFRVDQLIEQVIKQ 232
Sp1901          142 VELKDCLVQDKETQVIANRLAELL-TYHQIPIITDERKVLGVRTIMVRRARKTGQVQIIIV-TNR--QLNLTQLVKELVKD 217
B.subtilis YefA 161 IDMSACLIQSKNDEAVQAVKIDICANYGVKAYNEERHKGLRHLIMVRYGVVTGEMMIVFI-TRTSDFPHKAKIIEDITAQ 239

E.coli RlmC      162 LPOLKVITVNIQPVHMAIMEGETEYILTEQQALAERFNDVPLWIRPQSFQCTNPAVASQLYATARDWVRQLPVKHMWDLF 241
Sp1029          233 FPEIVSVMONINDQNTNAIFCKEWRTLYGQDYITDQMLGNDFQIAGPAFYQVNTFEMAERLYQTAIDFAELKKDDVIIDAY 312
Sp1901          218 FPEVVTVAVNTTAKTSELYEKEEIIWQESIQEGVLNVEFSLSPRAFVQLNPEQTEVLYSBAVKALDVDKEDHLIDAY 297
B.subtilis YefA 240 FPHVKSIVQNIINPNKTNVIFQNETNVIWGEYIYDLIGDVKFAISARSFYQVNPQTKVLYDKALEYAELOQEETVIDAY 319

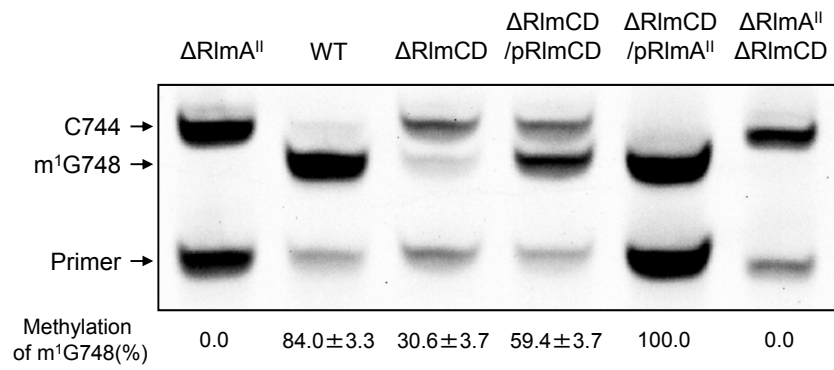
E.coli RlmC      242 CAVCGFGLHCAIPDMQLTGLEIASFAIACAKQSAELGLTRLQFQALDSTQFATA--QCDVPELVLVNPPRRGIGKPLC 318
Sp1029          313 SCIGTIGLSVAKHVKEVYGVLEIPEAVENSQKNASLNKIITNAHYVCDTAENAMKWLKEGICQPTVILVDPKRLTESFI 392
Sp1901          298 CGVGTIGFAPAKKVKTLRCMDIIEPAIEDAKRNAKRMGFDNTHYEAGTAEIIPRWYKEGYRADALIVDPPRIGLDDKLL 377
B.subtilis YefA 320 CGIGTISLFLAKQAKKVYGVLEIPEAIEDAKRNAELNGNTNAEFAVGEAEIVIPKWYEEGITADTLVVDPPRIGKCDREAL 399

E.coli RlmC      319 DYLSMAPRFIIYSSCNAQTMAKDIRELP--GFRIERVQLFDMFPHTAHYEVLTLLVKQ----- 375
Sp1029          393 KASAQTGADRIAYISCNVATMARDIKLYQELGYELKKVQPVLDLFPQTHHVEVTVALLSKLDVDKHISVEIELDEMDELTSAE 472
Sp1901          378 DTILTYVPEKMYIISCNVSTLARDLVRLEVE-YDLHYIQSVDMPHTARTEAVVKLIKKV----- 436
B.subtilis YefA 400 RTIVEMKPKRVVYVSCNPGTLARDLRVLEDDGGYVTRVQPVDMFPHTNHVECCVLIKKE----- 459

E.coli RlmC      375 ----- 375
Sp1029          473 SKATYAQIKEYVWNKFKLVSTLYIAQIKKCGLELREHYNKSKKDKQIIPQCTPEKEEAIMDALRHFKMI 543
Sp1901          436 ----- 436
B.subtilis YefA 459 ----- 459

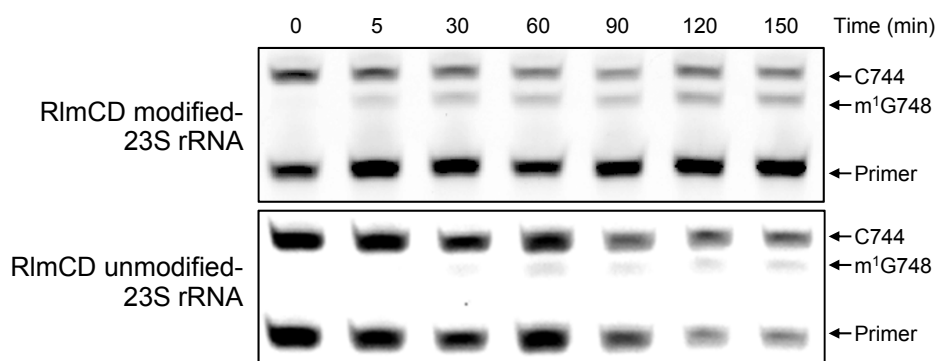
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Figure S2 Level of m¹G748 in 23S rRNAs prepared from the parent strain TIGR4 or its derivatives.



rRNAs prepared from strains TIGR4 (WT), Sp303 ($\Delta RlmA^{II}$), Sp332 ($\Delta RlmCD$), Sp360 ($\Delta RlmCD/pRlmCD$), Sp369 ($\Delta RlmA^{II}\Delta RlmCD$) and Sp370 ($\Delta RlmCD/pRlmA^{II}$) were sequenced by reverse transcriptase extension.

Figure S3 Time-course of m¹G748 formation in 23S rRNA catalyzed by 10nM RlmA^{II}.



rRNAs (10nM) prepared from strains Sp303 (RlmCD modified-23S rRNA; upper panel) and Sp369 (RlmCD unmodified-23S rRNA; lower panel) were catalyzed by 10nM RlmA^{II}. The methylation was stopped using phenol solution at different times and the level of m¹G748 was analyzed by reverse transcriptase extension.