SUPPORTING INFORMATION

A New Pathway for α-Ribazole-Phosphate Synthesis in *Listeria innocua*.

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TABLES

Table S1. Strains and plasmids used in this study

Strain or plasmid	Marker(s)[a]	Relevant genotype	Reference or source[b]
<u>*L. innocua* strain:</u>			
DD680			K. Boor[c]
<u>*S. enterica* strains:</u>			
TR6583		*metE205 ara-9* derivative of strain LT2	K. Sanderson[d] *via* J.R. Roth[e]
JE1244	Tc^R	*metE205 ara-9 cobT10*::Tn*10*d(*tet*$^+$)	lab collection
JE2701	Tc^R	*metE205 ara-9* DEL*cob291 cobB1176*::Tn*10*d(*tet*$^+$)	lab collection
JE8511	Tc^R Ap^R	*metE205 ara-9 cobT10*::Tn*10*d(*tet*$^+$) / pBAD24	
JE8612	Tc^R Ap^R	*metE205 ara-9 cobT10*::Tn*10*d(*tet*$^+$) / pCOBT48	
JE12331	Tc^R Ap^R	*metE205 ara-9* DEL*cob291 cobB1176*::Tn*10*d(*tet*$^+$) / pBAD24	
JE12332	Tc^R Ap^R	*metE205 ara-9* DEL*cob291 cobB1176*::Tn*10*d(*tet*$^+$) / pCOBT48	
JE12334	Tc^R Ap^R	*metE205 ara-9* DEL*cob291 cobB1176*::Tn*10*d(*tet*$^+$) / pCBLTS1	
JE12550	Tc^R Ap^R	*metE205 ara-9 cobT10*:: Tn*10*d(*tet*$^+$) / pCBLT1	
JE12552	Tc^R Ap^R	*metE205 ara-9 cobT10*::Tn*10*d(*tet*$^+$) / pCBLTS1	
JE12588	Tc^R Ap^R	*metE205 ara-9 cobT10*::Tn*10*d(*tet*$^+$) / pCBLS4	
<u>*E. coli* strains:</u>			
DH5α/F'		F' / *endA1 hsdR17* ($r_k^- m_k^+$) *glnV44 thi-1 recA1 gyrA* (Nal^R) *relA1* Δ(*lacIZYA-argF*)*U169*	(Woodcock *et al.*, 1989, Raleigh *et al.*, 1989)

		deoR (ϕ80*dlac*Δ(*lacZ*)*M15*)	
BL21(DE3)		F⁻ *ompT gal dcm lon hsdSB*(r_B^- m_B^-) λ(DE3 [*lacI lacUV5*-T7 gene 1 *ind1 sam7 nin5*])	Novagen
Plasmids:			
pGEM®-T Easy	Ap^R	TA cloning vector	Promega
pBAD24	Ap^R	cloning vector with P_{BAD} arabinose-inducible promoter	(Guzman *et al.*, 1995)
pTEV5	Ap^R	TEV protease-cleavable His_6 tag overexpression vector	(Rocco *et al.*, 2008)
pKLD116	Ap^R	TEV protease-cleavable His_6 / maltose-binding protein tag overexpression vector	(Rocco *et al.*, 2008)
pCOBT48	Ap^R	*S. enterica cobT*⁺	lab collection
pCBLT1	Ap^R	*L. innocua cblT*⁺	
pCBLS3	Ap^R	*L. innocua cblS*⁺ translational fusion to His_6 / maltose-binding protein tags for protein purification	
pCBLS4	Ap^R	*L. innocua cblS*⁺	
pCBLS5	Ap^R	*L. innocua cblS*⁺ translational fusion to His_6 tag for protein purification	
pCBLTS1	Ap^R	*L. innocua cblT*⁺ *cblS*⁺	

[a] Tc^R, tetracycline resistance; Ap^R, ampicillin resistance.
[b] Unless otherwise indicated, all strains and plasmids were constructed during the course of these studies.
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[e] Section of Microbiology, College of Biological Sciences, University of California-Davis

Table S2. Composition of MLM (minimal *Listeria* medium)

Component	Concentration (mM)
fructose	100
4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES, pH 7.0)	20
$MgSO_4$	2
adenine HCl	0.5
$CaCl_2$	0.1
	Concentration (g l^{-1})
$Na_2HPO_4 \cdot 7H_2O$	6.8
KH_2PO_4	3
NaCl	0.5
	Concentration (mg l^{-1})
cysteine	10
leucine	10
isoleucine	10
valine	10
riboflavin	5
thiamine	1
nicotinamide	1
pyridoxal	1
p-aminobenzoic acid	1
biotin	0.5
$FeCl_2$	0.135
$ZnCl_2$	0.017
$MnCl_2 \cdot 4H_2O$	0.01
$CoCl_2 \cdot 6H_2O$	0.006
$NaMoO_4 \cdot 2H_2O$	0.006
α-lipoic acid	0.005
$CuCl_2 \cdot 2H_2O$	0.005
Na_2SeO_4	0.005

Table S3. Comparison of the presence of *cobT*, *bluB*, and *cbiZ* homologs in bacterial genomes encoding *cblS* or *cblT* homologs

Species[a]	**Locus tag of predicted *cblT* homolog (% identity, % similarity to CblT from *L. innocua*)**[b]	**Locus tag of predicted *cblS* homolog (% identity, % similarity to CblS from *L. innocua*)**[c]	**Locus tag of predicted *cobT* homolog(s) (% identity, % similarity to CobT from *S. enterica*)**[d]	**Locus tag of predicted *bluB* homolog (% identity, % similarity to BluB from *R. rubrum*)**[g]	**Locus tag of predicted *cbiZ* homolog (% identity, % similarity to CbiZ from *M. mazei*)**[h]
Alkaliphilus metalliredigens QYMF	Amet_0066 (41%, 58%)	Amet_0067 (34%, 54%)	Amet_0464 (45%, 62%)		
Alkaliphilus oremlandii OhILAs	Clos_2207 (33%, 56%)	Clos_2206 (36%, 58%)			
Anaerotruncus colihominis DSM 17241		ANACOL_03809 (35%, 55%)	ANACOL_02043 (41%, 61%)		
Anoxybacillus flavithermus WK1	Aflv_1051 (33%, 53%)	Aflv_1052 (28%, 48%)	Aflv_2169 (42%, 59%)		Aflv_1043[i] (28%, 50%)
Bacillus coahuilensis m4-4	Bcoam_010100007990 (30%, 47%)				Bcoam_010100007955[i] (25%, 46%)
Bacillus halodurans C-125		BH0853 (33%, 54%)	BH0284 (40%, 60%)		BH1587[i] (34%, 50%)
Carboxydothermus hydrogenoformans Z-2901	CHY_0775 (37%, 53%)	CHY_0776 (32%, 51%)	CHY_0480 (38%, 61%)		
Clostridium botulinum A Hall	CLC_1007 (43%, 71%)	CLC_0877 (32%, 53%)			
Clostridium perfringens 13	CPE1308 (42%, 69%)	CPE1307 (26%, 50%)	CPE1034 (38%, 60%)		
Clostridium sporogenes ATCC 15579	CLOSPO_01660 (42%, 71%)	CLOSPO_01525 (31%, 52%)			
Clostridium tetani E88	CTC00741 (42%, 64%)	CTC00742 (36%, 58%)	CTC02290 (39%, 60%)		
Desulfitobacterium hafniense Y51		DSY2725 (34%, 50%)	DSY2114 (42%, 61%)		
Geobacillus kaustophilus HTA426	GK2256 (33%, 51%)	GK2255 (30%, 45%)	GK1793 (40%, 57%)		GK2264[i] (29%, 48%)

Geobacillus thermodenitrificans NG80-2	GTNG_2189 (32%, 50%)	GTNG_2188 (30%, 46%)	GTNG_1683 (40%, 57%)		GTNG_2197[i] (27%, 47%)
Heliobacterium modesticaldum Ice1	HM1_1580 (33%, 51%)	HM1_1579 (34%, 54%)	HM1_2401 (41%, 62%)		HM1_1855 (27%, 43%)
Lactobacillus brevis gravesensis ATCC 27305	HMPREF0496_0555 (47%, 68%)	HMPREF0496_0556 (42%, 59%)			
Lactobacillus buchneri ATCC 11577	HMPREF0497_0511 (47%, 67%)	HMPREF0497_0512 (43%, 60%)			
Lactobacillus hilgardii ATCC 8290	HMPREF0519_1876 (54%, 70%)	HMPREF0519_1306 (42%, 60%)			
Listeria innocua Clip11262	lin1153 (100%, 100%)	lin1110 (100%, 100%)			
Listeria monocytogenes EGD-e	lmo1190 (97%, 98%)	lmo1146 (81%, 87%)			
Listeria welshimeri sv 6b SLCC5334	lwe1147 (94%, 98%)	lwe1104 (78%, 88%)			
Lysinibacillus sphaericus C3-41	Bsph_2450 (31%, 50%)	Bsph_2451 (26%, 47%)	Bsph_1664 (48%, 64%)	Bsph_2918 (33%, 49%)	Bsph_0967[i] (25%, 45%)
Moorella thermoacetica ATCC 39073	Moth_0191 (48%, 69%)	Moth_0190 (35%, 51%)	Moth_1101 (41%, 63%)		
			Moth_1721 (41%, 59%)		
Natranaerobius thermophilus JW/NM-WN-LF	Nther_0938 (45%, 68%)	Nther_0939 (33%, 54%)			
Propionibacterium acnes KPA171202		PPA0108 (27%, 46%)	PPA0441[e] (40%, 57%)	PPA0953[f] (38%, 53%)	
			PPA0953[f] (33%, 50%)		
Symbiobacterium thermophilum IAM 14863	STH1931 (41%, 54%)	STH1930 (35%, 55%)			
Thermoanaerobacter brockii subsp. *finnii* Ako-1	ThebrDRAFT_1429 (34%, 57%)	ThebrDRAFT_1430 (33%, 53%)			
Thermoanaerobacter ethanolicus CCSD1	TeCCSD1DRAFT_0616 (33%, 56%)	TeCCSD1DRAFT_0615 (35%, 54%)			
Thermoanaerobacter italicus Ab9	ThitDRAFT_083	ThitDRAFT_083			

	7 (33%, 55%)	6 (33%, 54%)			
Thermoanaerobacter mathranii subsp. *mathranii* str. A3	TmathDRAFT_1629 (34%, 55%)	TmathDRAFT_1628 (33%, 54%)			
Thermoanaerobacter pseudethanolicus ATCC 33223	Teth39_1898 (34%, 57%)	Teth39_1897 (33%, 53%)			
Thermoanaerobacter tengcongensis MB4	TTE0378 (37%, 57%)	TTE0379 (36%, 53%)			TTE2426 (44%, 65%)
Thermoanaerobacterium thermosaccharolyticum DSM 571	TtheDRAFT_2239 (35%, 55%)	TtheDRAFT_2240 (32%, 50%)			

[a] For species with multiple sequenced strains, a single representative example has been selected.
[b] Identified by BLAST search of the Integrated Microbial Genomes database (http://img.jgi.doe.gov), accessed March 23, 2010 (Markowitz *et al.*, 2006).
[c] Identified by BLAST search of the Integrated Microbial Genomes database (http://img.jgi.doe.gov), accessed March 23, 2010 (Markowitz *et al.*, 2006).
[d] Identified by BLAST search of the Integrated Microbial Genomes database (http://img.jgi.doe.gov), using the *Salmonella enterica* CobT sequence (Trzebiatowski *et al.*, 1994) as the search term, accessed June 18, 2010 (Markowitz *et al.*, 2006). Percent identity and percent similarity were calculated with the BLAST 2 Sequences program (McGinnis & Madden, 2004).
[e] This gene encodes a fusion protein between CobT and a CobU (adenosylcobinamide kinase / adenosylcobinamide-phosphate guanylyltrasnferase) ortholog (Markowitz *et al.*, 2006).
[f] This gene encodes a fusion protein between CobT and a BluB (aerobic DMB synthase) ortholog (Markowitz *et al.*, 2006).
[g] Identified by BLAST search of the Integrated Microbial Genomes database (http://img.jgi.doe.gov), using the *Rhodospirillum rubrum* BluB sequence (Gray & Escalante-Semerena, 2007) as the search term, accessed April 8, 2010 (Markowitz *et al.*, 2006).
[h] Identified by BLAST search of the Integrated Microbial Genomes database (http://img.jgi.doe.gov), using the *Methanosarcina mazei* CbiZ sequence (Gray *et al.*, 2008) as a search term, accessed April 8, 2010 (Markowitz *et al.*, 2006).
[i] These genes encode fusion proteins between CbiZ and homologs of BtuD, the ATPase component of the Btu corrinoid-specific ABC transport system (Gray *et al.*, 2008; Markowitz *et al.*, 2006).

Table S4: Primers used in this study

Primer	Sequence[a]
[1]	CTG TTT CTC CAT ACC CGT T
[2]	GGC TGA AAA TCT TCT CTC AT
[3]	ACT GAA GAA TTC ATG AA ATT CAA AAA TTA GTA TTA TGT GCG
[4]	ACT GAA TCT AGA TTA GTA CCC GGC AAT TCG TCT T
[5]	GAC GTC CCG GGC TAG CAT GCC TCA AGT GAG GGA TTT
[6]	GTG CGG CCG CAA GCT TTC AGC CAT GGT AAT TCC TCA A
[7]	CGA GCG GAA CCG CCT CG
[8]	CCA TTC GCC AAT CCG GAT
[9]	TCG TAC TAC CAT CAC CAT CAC
[10]	CTA GCA GGA GGA ATT CCA CCA TGC CTC AAG TGA GGG ATT T
[11]	CAA AAC AGC CAA GCT TTC AGC CAT GGT AAT TCC TCA A
[12]	CTA GCA GGA GGA ATT CTT GAA GAT TCA AAA ATT AGT ATT ATG TGC G
[13]	CTT GAG GCA TTC TAG ATT AGT ACC CGG CAA TTC GTC TT
[14]	CGG GTA CTA ATC TAG AAT GCC TCA AGT GAG GGA TTT
[15]	CCA AGC TTG CAT GCC TGC AGT CAG CCA TGG TAA TTC CTC AA

[a] Restriction sites introduced for cloning are underlined.

FIGURES

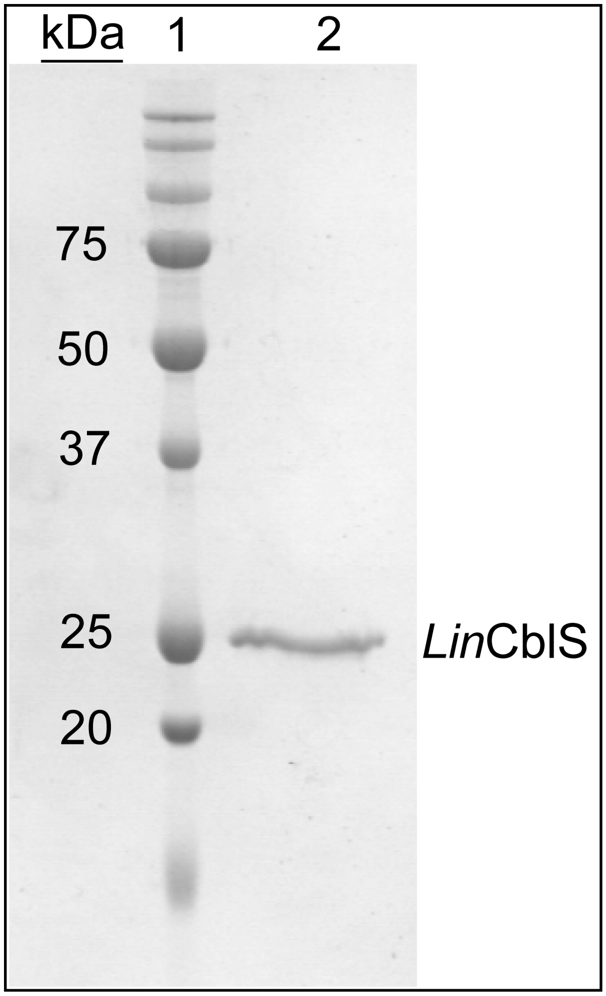

Figure S1. Purification of *Lin*CblS protein. Purified *Lin*CblS (2 μg) was resolved by 12% SDS-PAGE (Laemmli, 1970) and stained with Coomassie Blue (Sasse, 1991). Lane 1. Electrophoretic mobility of molecular mass markers (Precision Plus Protein™ Standards, Bio-Rad Laboratories). Molecular mass in kilodaltons (kDa) is indicated. Lane 2. *Lin*CblS protein, > 98% homogenous.

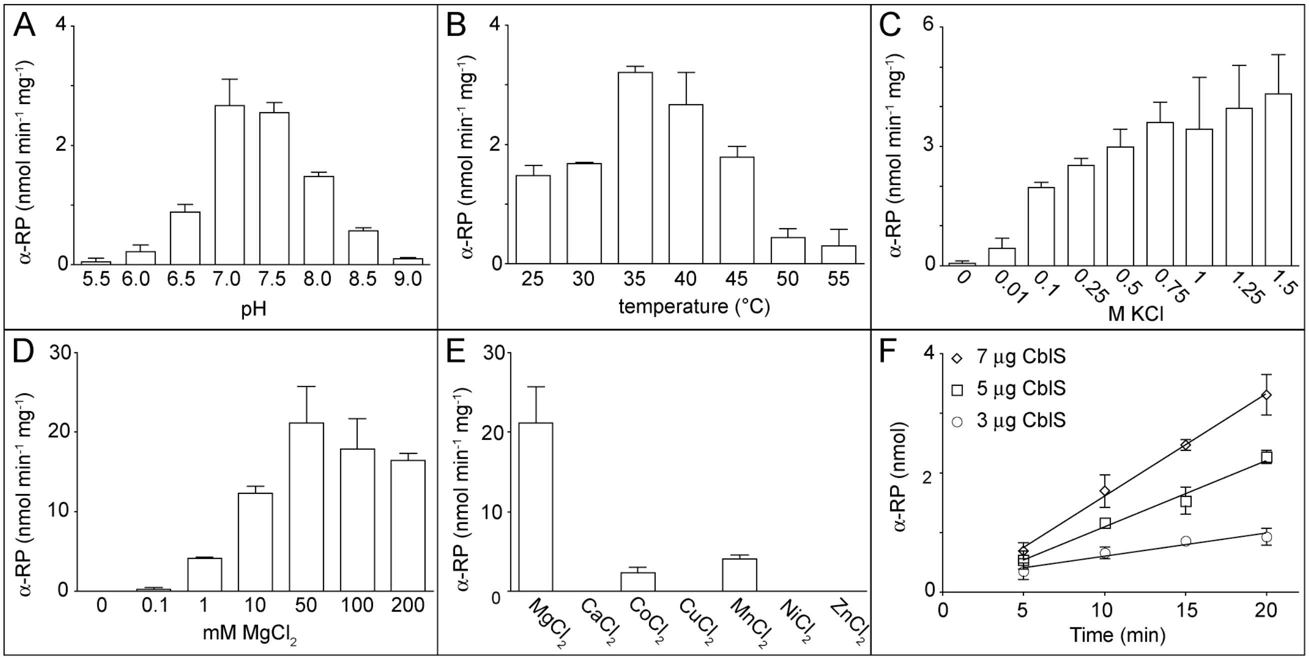

Figure S2. Optimization of *Lin*CblS reaction conditions. α-R kinase activity of *Lin*CblS (expressed as nanomoles of α-RP min^{-1} mg^{-1} protein) is shown, with error bars of one standard deviation. Unless indicated, reactions contained Tris-HCl buffer (100 mM, pH 7.0), TCEP (5 mM), KCl (0.75 M), $MgCl_2$ (50 mM), α-R (30 µM), *Lin*CblS (3 - 10 µg) and ATP (1 mM) and were incubated at 35°C. **A**. *Lin*CblS activity as a function of pH at 37°C, 0.5 M KCl, 1 mM $MgCl_2$. **B**. *Lin*CblS activity as a function of temperature at 0.5 M KCl, 1 mM $MgCl_2$. **C**. *Lin*CblS activity as a function of KCl concentration at 1 mM $MgCl_2$. **D**. *Lin*CblS activity as a function of $MgCl_2$ concentration. **E**. *Lin*CblS activity in the presence of different divalent cations (50 mM). **F**. Product formation as a function of time and *Lin*CblS concentration at 60 µM α-R.

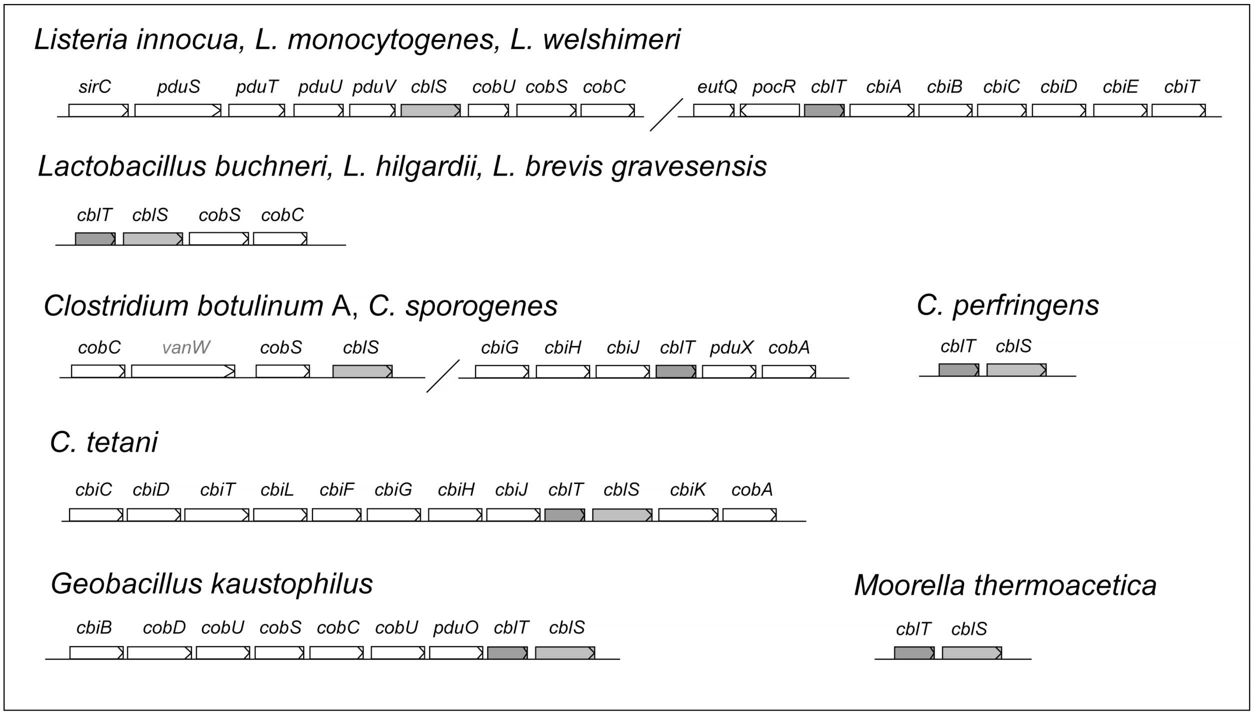

Figure S3. Genomic context of *cblT* and *cblS* homologs in selected bacteria. The organization of genes encoding products predicted to be involved in coenzyme B_{12} biosynthesis or utilization near predicted *cblT* (dark grey) and *cblS* (light grey) homologs in representative bacterial genomes is indicated. Arrows within boxes indicate the direction of transcription of the indicated gene. Diagonal slashes indicate gaps between *cblT*- and *cblS*-associated loci in a single genome. The products of the indicated genes are as follows (the locus tags of the *Listeria innocua* Clip11262 homolog of each gene is provided in parentheses): *sirC* (*lin1105*), precorrin-2 dehydrogenase; *pduS* (*lin1106*) and *pduV* (*lin1107*), predicted 1,2-propanediol utilization proteins; *pduT* (*lin1108*) and *pduU* (*lin1109*), metabolosome shell proteins involved in 1,2-propanediol utilization; *cblS* (*lin1110*), α-R kinase; *cobU* (*lin1111*), bifunctional AdoCbi kinase / GTP:AdoCbi-P guanylyltransferase; *cobS* (*lin1112*), AdoCbl-P synthase; *cobC* (*lin1113*), AdoCbl-5'-P phosphatase; *eutQ* (*lin1151*), predicted ethanolamine utilization protein; *pocR* (*lin1152*), putative 1,2-propanediol-sensing transcription factor; *cblT* (*lin1153*), α-R transporter; *cbiA* (*lin1154*), cobyrinic acid *a,c*-diamide synthase; *cbiB* (*lin1155*), AdoCbi-P synthetase; *cbiC* (*lin1156*), precorrin-8X methylmutase; *cbiD* (*lin1157*), cobalt-precorrin-6A synthase; *cbiE* (*lin1158*), precorrin-6B methylase; *cbiT* (*lin1159*), precorrin-8W decarboxylase; *vanW*, vancomycin B-type resistance protein (*C. botulinum* locus tag CLC_0875, no *L. innocua* homolog and no known role in coenzyme B_{12} metabolism); *cbiG* (*lin1161*), cobalt-precorrin-5A hydrolase; *cbiH* (*lin1162*), precorrin-3B C17-methyltransferase; *cbiJ* (*lin1163*), precorrin-6X reductase; *pduX* (*lin1134*), L-threonine kinase; *cobA* (*C. tetani* locus tag CTC00744, no *L. innocua* homolog), ATP:corrinoid adenosyltransferase; *cbiL* (*lin1166*), precorrin-2 methyltransferase; *cbiF* (*lin1160*), precorrin-4 C-11 methyltransferase; *cbiK* (*lin1165*), cobalt chelatase; *pduO* (*lin1128* and *lin1172*), ATP:corrinoid adenosyltransferase.

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A. metaliredigens           --------------------------------------------------
A. oremlandii               --------------------------------------------------
A. flavithermus             --------------------------------------------------
B. coahuilensis             --------------------------------------------------
C. hydrogenoformans         --------------------------------------------------
C. botulinum                --------------------------------------------------
C. perfringens              --------------------------------------------------
C. sporogenes               --------------------------------------------------
C. tetani                   --------------------------------------------------
G. kaustophilus             --------------------------------------------------
G. thermodenitrificans      --------------------------------------------------
H. modesticaldum            ---------------------------------------------MSNMP 5
L. gravesensis              --------------------------------------------------
L. buchneri                 --------------------------------------------------
L. hilgardii                --------------------------------------------------
L. innocua                  --------------------------------------------------
L. monocytogenes            --------------------------------------------------
L. welshimeri               --------------------------------------------------
L. sphaericus               --------------------------------------------------
M. thermoacetica            ---------------------------------------------MAKPE 5
N. thermophilus             ---------------------------------------------MLS-- 3
S. thermophilum             MPLEHLTTTSGIGAPKHRCTVACRTGTGESRAQRGAAEYGHNGVPTKRLL 50
T. brockii                  --------------------------------------------------
T. ethanolicus              --------------------------------------------------
T. italicus                 --------------------------------------------------
T. mathranii                --------------------------------------------------
T. pseudethanolicus         --------------------------------------------------
T. tengcongensis            --------------------------------------------------
T. thermosaccharolyticum    --------------------------------------------------

A. metaliredigens           -MISRNRKKQMDTKMLVKMAMLIALSGIGAMIKIQG---SIALDAVPGFY 46
A. oremlandii               -MNNTKNVSMSNIQVITRSGLLIGLSAIGAMIKIQG---TIAFDSMPGFF 46
A. flavithermus             ------------MRRFTLLVMFISLSVIGSLIKIPFTIGSIALDSAPAVV 38
B. coahuilensis             ----------MKTSRVNLIAAFIALSVIGGFIKIPSPISSIALDSVAPLI 40
C. hydrogenoformans         ----------MNIRTLTTLAILIALSAVGAFIKIPSPTGTVALDSLPGYF 40
C. botulinum                ----------MKTKRLVIMGLFIALSFVGANIKIMG---SIAFDSMPAFL 37
C. perfringens              -MNFTKEKKRLDVKSLCFSAILIAISVILANFPIFS---SIALDSMPAFV 46
C. sporogenes               ----------MKTKRLVIVGLFIALSFVGANIKIMG---SIAFDSMPAFL 37
C. tetani                   ----------MKTNKMVITALFIALSFIGANIKIMG---TVAFDAMPGFL 37
G. kaustophilus             -----------MNRRLAWTAVCLALSAIGSFIKLPTFVGSIALDSAPALV 39
G. thermodenitrificans      -----------MNRRFVWLAVCMALSVIGSFIKLPTFVGSIALDSAPALV 39
H. modesticaldum            TQYNEVGPSWKSVRSIALMGLLIALSAVGAMVKLPSPVGTIGLDSAPGFF 55
L. gravesensis              -------MRNDYLKKLMLTTIFLALCVVGANVKILG---SIALDSAPAFL 40
L. buchneri                 -------MRNDYLKKLMLTTIFLALCVVGANVKILG---SIALDSAPAFL 40
L. hilgardii                ------MTQSDRLNRLTLAAMLLALCVIGANVKIMG---SVAFDSAPAFL 41
L. innocua                  ----------MKIQKLVLCAMLIAMCVIGANIKLMG---SVAFDAAPAFI 37
L. monocytogenes            ----------MKIQKLVLCAMLIAMCVIGANIKLMG---SVAFDAAPAFI 37
L. welshimeri               ----------MKIQKLVLCAMLIAMCVIGANIKLMG---SVAFDAAPAFI 37
L. sphaericus               -------MDRQTLMTWILTAMVAAICAVGAAIKVPAFISTAALDSAPAFL 43
M. thermoacetica            LSSPAVKASRWTARRLATLAMLIALSTVGANLKIPSITGTPAFDSFPGFL 55
N. thermophilus             ----------LDHRELVKIALLIALSMIGSQIKIPSLTGTPALDSFPAYL 43
S. thermophilum             LGRGGLLVLLARTNTLVRLGILLALSVLGAYIKLGP--SSIAFDAMAGFV 98
T. brockii                  -MK-EKANALKNVKTLTLVAMLIALSAVGALIKIFN---TVAFDSMPGYF 45
T. ethanolicus              -MK-EKANALKNVKTLTLVAMLIALSAVGALIKIFN---TVAFDSMPGYF 45
T. italicus                 -MK-EKANALKNVKTLTLVAMLIALSAVGALIKIFN---TVAFDSMPGYF 45
T. mathranii                -MK-EKANTLKNVKTLTLVAMLIALSAIGALIKVFN---TVAFDSMPGYF 45
T. pseudethanolicus         -MK-EKANALKNVKTLTLVAMLIALSAVGALIKIFN---TVAFDSMPGYF 45
T. tengcongensis            -MK-EKEVSLGNVKTLTLVAMLIALSAVGALIKIFN---TVAFDSMPGYF 45
T. thermosaccharolyticum    -MN-TKTTTIKNVKTLTLVAMLIAMSAVGAMIKIYN---TVAFDSLPGYF 45

A. metaliredigens           AALLLGPMAGGIVAFAGHLISALTAGFPMTVPMHLVVAVEMFIIVALFSV 96
A. oremlandii               AALFISPMAGGAVASLGHLLTAFTSGFPLTLPMHLMLTVVMGIIAYFFGV 96
A. flavithermus             AAVLLGPTAAAVVASVGHVVSALFGGFPLG-PFHILVAIEMAALLYIVGL 87
B. coahuilensis             AASMFGGMIGGFIGSIGHLLSSLSGGFPLG-PFHFIIMIEMFCILFIYAL 89
C. hydrogenoformans         AALYLSPGLGALVAALGHLLSAATAGFPLTLPLHLLVALEMAIFAAVFGV 90
C. botulinum                GTLILGPIMGAIIGAVAHFLSALTSGFPLSLPVHMIVMVDMAVTMILFGI 87
C. perfringens              GGIIISPVVGGIIGLLAHLFVALRTGFPLSLPVHILVALEMFVVVYITSI 96
C. sporogenes               GTLILGPIMGAIIGAVAHFLSALTSGFPLSLPVHMIVMVDMAVTMLLFGV 87
C. tetani                   GALLLGPVYGGIIGGVGHFLTALTSGFPLSLPVHLVITVIMAATMAIFAL 87
G. kaustophilus             AAGVLGPRAGAAVAGLGHLVSALIGGFPLG-PVHWFVALEMAGLGALFAV 88
G. thermodenitrificans      AAAVLGPQAGATVAGLGHFISAYIGGWPLG-PFHWLVACEMAVLGALFAA 88
H. modesticaldum            AALALGATGGMIVIALGHLLTAMVVGFPLTLPVHLFIALQMAMWAYVFRR 105
L. gravesensis              GAILLGPAIGAFLGFFGHLLSAMLAGFPLTLPVHLIIGVMMAACMFVFGL 90
L. buchneri                 GAILLGPAIGAFLGFFGHLLSAMLAGFPLTLPVHLIIGVMMAACMFVFGL 90
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L. hilgardii                 GAVLLGPWFGATLGLFGHLVSAALAGFPLTLPIHLIIGVAMGICMLIFGL 91
L. innocua                   GTLLLGPMYGAVLGIFGHLTSALLAGFPLTLPIHLIVAGMMGVTMIAYGF 87
L. monocytogenes             GTLLLGPMYGAVLGIFGHLTSALLAGFPLTLPIHLIVAGMMGVTMIAYGF 87
L. welshimeri                GTLLLGPMYGAVLGIFGHLTSALLAGFPLTLPIHLIVAGMMGVTMIVYGF 87
L. sphaericus                GVVFLSPLLAGIGGFIGHFISALTAGFPLG-PLHVIIAVEMFIVVWIFGI 92
M. thermoacetica             GALILGPADGALIAALGHLLTAFTAGFPLTPPLHLVIAAGMAAVVALFAI 105
N. thermophilus              AAFIWVGKYAAAIGFFGHIFTSLLVGFPMSIPIHLILAVGMAGCVYAVSH 93
S. thermophilum              AALLMGPAAGALICGLGHVAVAAVTGFPLTLPFHLASAAAMAGVGCLGGL 148
T. brockii                   AALYLGSWYGALVISLGHMLTAITSGFPLGLTNHIYIAVQMGLYAYLFKF 95
T. ethanolicus               AALYLGSWYGALVISLGHMLTAITSGFPLGLTNHIYIAVQMALYAYLFKF 95
T. italicus                  AALYLGSWYGALVISLGHMLTAITSGFPLGLTNHIYIAVQMALYAYLFRF 95
T. mathranii                 AALYLGGWYGALVISLGHMLTAITSGFPLGLTNHIYIAVQMALYAYLFRF 95
T. pseudethanolicus          AALYLGSWYGALVISLGHMLTAITSGFPLGLTNHIYIAVQMGLYAYLFKF 95
T. tengcongensis             AALYLGSLYGAIVIALGHLLTAVTSGFPLGVLNHLYIALQMAVYAYLFRV 95
T. thermosaccharolyticum     ASLYFGSYIGAIVISLGHIFTALTSGFPLGIPNHIIIAVSMAVCAYFYSI 95

A. metaliredigens            VW-------QKINPWVAIIVGILLNGVGAGLLVVPMSILLGLPLNGWALF 139
A. oremlandii                IE-------RKVNGVLACVIAILLNGPVATFIAGITASLLGLPLSGSAMF 139
A. flavithermus              LIR------RGWFIFSYVCFFIG-NALLAPLPFV-------WIMS-PAFV 122
B. coahuilensis              FYK------TNKWV--AFLFFIGANTFLAPLPFL-------FFHGPIIFI 124
C. hydrogenoformans          LG-------KRN-VIVGIVVATLLNGVIAPLSFAI------MPKFGMAFF 126
C. botulinum                 VYNKFKNKNNILAAIVATVVAVIINGPVSVFAI--------IPIAGKG-V 128
C. perfringens               IF-------NRGKVILAGIVGTLLNGIVFTLITGVFMYFVLGGMNPIDFL 139
C. sporogenes                VYNKLKNKNNILAAIVATIVAVIINGPVSVFAI--------IPITGKG-V 128
C. tetani                    VYKVLVKKNKVLAMILAIIVGVTVNGPINLLVLTPLL----MPIMGKAGI 133
G. kaustophilus              LHR------RGWKIGSAVVFFIG-NAFLAPLPLA-------VSFG-WPFV 123
G. thermodenitrificans       MYG------RGWRFGGAIAFFVG-NVFLAPLPLV-------IAFG-WPLV 123
H. modesticaldum             VN-------GQFGLTAAFAAAVCLNGVVSSLTM--------LLLGGWGAV 140
L. gravesensis               LRQRLN-LSRGLVIVISDAIGYLINVPLELTLL--------YPILKQS-V 130
L. buchneri                  LRQRLT-LSRGLVIVISDAIGYLINVPLELTLL--------YPILKQS-V 130
L. hilgardii                 VRKWLG-KDTLKGVLTSDVLGYAINVPIELVLL--------YPLMKQA-V 131
L. innocua                   TRHKLAEKNQLVAISASSVVAFVFNCPLSLLAL--------YPLMHQA-V 128
L. monocytogenes             TRQKLAEKNQLVAISVSSIVAFVFNCPLSLLAL--------YPLMHQA-V 128
L. welshimeri                TRQKLADKNQLIAVSVSSMVAFIFNCPLSLLAL--------YPLMNQA-V 128
L. sphaericus                MHK------KGMHFWKWPVALVL-NGIVAPLPFY-------FIIS-PAFF 127
M. thermoacetica             FY-------RFS-PWLGIAAGIALNGLLLPALFIP------LPGFGKAFF 141
N. thermophilus              VN-------NYSGRAAGVLAGMVLNGLILPGIFVL------IPGFGIPFF 130
S. thermophilum              AA-------RRFGLVAGAAVLVVANGILAPALLALLP-----NPLGLGLF 186
T. brockii                   FY-------RKFNIYIAVIAATILNGPVATLLF--------VPIFGWGFF 130
T. ethanolicus               FY-------QKFNIYIAVIAATILNGPVATLLF--------VPIFGWGFF 130
T. italicus                  FY-------RKFNIYIAVIVATILNGPVATLLF--------VPIFGWGFF 130
T. mathranii                 FY-------RKFNIYIAVIVATILNGPVATLLF--------VPIFGWGFF 130
T. pseudethanolicus          FY-------RKFNIYIAVIAATILNGPVATLLF--------VPIFGWGFF 130
T. tengcongensis             FY-------KKFNVYVAIVLTTLLNGPLATLLF--------VPMFGWGFF 130
T. thermosaccharolyticum     AY-------KKFNSYVAVAVGTILNGPVATLIF--------VPQYGWGFF 130

A. metaliredigens            AVIWMPLLIGSTVNILIAASLYKIMGKGKSVNGN--------- 173
A. oremlandii                TALVIPLTVVAAVNVILAYIIFKVLPRK--------------- 167
A. flavithermus              LSIIPPLALATGVNLAIAVVVSKALQRAWGKQ-----HA---- 156
B. coahuilensis              FSLLPSLGIATVVNIILVIILLPKIQWIYSHRFSGTFQ----- 162
C. hydrogenoformans          TAMVVPLLVASFVNILLAGLTARALRGKTER------------ 157
C. botulinum                 LALLPILSLAALANVIIAIIIYRFIPEKYFQR----DK----- 162
C. perfringens               KLLGLPLTLASLVNIVVAFIVSKGLKNANI------------- 169
C. sporogenes                LAILPILSLAALANVIIAIIIYRFIPEKYFER----NK----- 162
C. tetani                    FALVPVLSGVAAINAVVAVLIYKFLPRSLKKY----ENK---- 168
G. kaustophilus              FAVIPPLSAAAAVNVLIALAVMPVVVRLAAKAGVEAPHA---- 162
G. thermodenitrificans       IAVIPPLSVATAMNILVAMAVMPSVVRLAAKVGGKAPHA---- 162
H. modesticaldum             MAVMPFLVAASAVNVGLAAAAYRGMKGIL-------------- 169
L. gravesensis               VALFLPLTIATVLNLILCELVSAALPKRVRRT-FKALKH---- 168
L. buchneri                  VALLLPLTIATILNLILCELVSAALPKRVRRT-FKALKH---- 168
L. hilgardii                 WAFFVPLTIATILNLIVCEVIYAALPHRVKDAPFLTPQK---- 170
L. innocua                   FVLFPVLAIGSICNIFVAEVVYQVLPERWKRR--IAGY----- 164
L. monocytogenes             FVLFPVLAIGSVCNIFVAEVVYQVLPERWKRR--IAGY----- 164
L. welshimeri                FVLFPVLAIGSICNIFVAEVVYQVLPERWKRR--IAGY----- 164
L. sphaericus                WGALGSIFIATAINLIIVAVVMPILSKVFVRKAGRV------- 163
M. thermoacetica             LAMVVPLLIASALNIVLAATAFTSLRRVFPASYAAGRGKGEGK 184
N. thermophilus              MGMVVPVTVASAVNIGLAVTAAPVCSQFLNRKPV--------- 164
S. thermophilum              AALALPLTVAAGANAAVALLVVLGLRRAGVEG----------- 218
T. brockii                   AAWVLPLTIASFANVFLAALVYKAIPKRSRE------------ 161
T. ethanolicus               AAWVLPLTIASFANVFLAALVYKTIPKRSRE------------ 161
T. italicus                  AAWVLPLTIASFANVFLAALIYKAIPKRSRE------------ 161
T. mathranii                 AAWVLPLTIASFANVFLAALIYKAIPKRSRE------------ 161
T. pseudethanolicus          AAWVLPLTIASFANVFLAALVYKAIPKRSRE------------ 161
T. tengcongensis             ITWVFPLTIASFINISLAALIYKILSKKEQ------------- 160
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T. thermosaccharolyticum	VQMVFPLTIASFANVFLAAVIFKAISTKIKR------------	161

Figure S4. Alignment of CblT homologs. ClustalW2 (Larkin *et al.*, 2007) alignment of predicted CblT proteins from 29 bacterial species (Table S4). Conserved residues are shaded. Putative transmembrane helices are underlined and were predicted from the *L. innocua* Clip11262 CblT protein sequence with the toppred algorithm (http://mobyle.pasteur.fr/cgi-bin/portal.py?form=toppred, accessed March 24, 2010) (von Heijne, 1992; Claros & von Heijne, 1994).

```
CblS        -------------------------MPQVRDLSVIDVPG--GCVLTSCDISAGFGEKVH 32
PurM        MTDKTSLSYKDAGVDIDAGNALVGRIKGVVKKTRRPEVMGGLGGFGALCALPQKYREPVL 60
ThiL        --------------MACGEFSLIARYFDRVRSSRLDVELG--IGDDCALLNIPEKQTLAI 44
HypE        -----------------MQQLINSLFMEAFANPWLAEQED--QARLDLAQLVAEGDRLAF 41
SelD        -MSENSIRLTQYSHGAGCGCKISPKVLETILHSEQAKFVD--PNLLVGNETRDDAAVYDL 57

CblS        DGLRVAPEVTARLTLRVALLEMLASGAVVVAVSDVIGAEMEPTGKRVIAGLKDELFKADL 92
PurM        VSGTDGVGTKLRLAMDLKRHDTIGIDLVAMCVNDLVVQGAEPLFFLDYYATGK--LDVDT 118
ThiL        STDTLVAGN--HFLPDID-PADLAYKALAVNLSDLAAMGADPAWLTLALTLPD--VDEAW 99
HypE        STDSYVIDP--LFFPGGN-IGKLAICGTAN---DVAVSGAIPRYLSCGFILEEG-LPMET 94
SelD        GNGTSVISTTDFFMPIVDNPFDFGRIAATNAISDIFAMGGKPIMAIAILGWPINKLSPEI 117

CblS        GHIELNGSTEENMNVTQTSVG-------------------VLVTGFATKAALKLINVHEA 133
PurM        ASAVISGIAEGCLQSGCSLVGGETAEMPGMYHGEDYDVAGFCVGVVEKSEIIDGSKVSDG 178
ThiL        LESFSDSLFDLLNYYDMQLIGGDTTRGP-------LSMTLGIHGFVPMGRALTRSGAKPG 152
HypE        LKAVVTSMAETARAAGIAIVTGDTKVVQRGAV-DKLFINTAGMGAIPANIHWGAQTLTAG 153
SelD        AREVTEGGRYACRQAGIALAGGHSIDAP------EPIFGLAVTGIVPTERVKKNSTAQAG 171

CblS        AVLFAFG---EPIVGAEVLQRMMEMPDYPLVKQLVSD---------------------- 167
PurM        DVLIALGSSGPHSNGYSLVRKILEVSGCDPQTTELDGKPLA--DHLLAPTRIYVKSVLE- 235
ThiL        DWIYVTGTPGDSAAGLAILQNRLQVADAKDADYLIKRHLRP--SPRILQG-QALRDLANS 209
HypE        DVLLVSGTLGDHGATILNLREQLGLDGELVSDCAVLTPLIQ--TLRDIPGVKALRDATR- 210
SelD        CKLFLTKPLGIGVLTTAEKKSLLKPEHQGLATEVMCRMNIAGASFANIEGVKAMTDVTG- 230

CblS        ---------SRVLEVVPVGSKGMAYEANTLARLNDCVFEASG------------------ 200
PurM        -------LIEKVDVHAIAHLTGGGFWENIPRVLPDNTQAVID-----ESSWQWPEVFNWL 283
ThiL        AIDLSDGLISDLGHIVKASDCGARIDLALLPFSDALSRHVEP---EQALRWALSGGEDYE 266
HypE        -----GGVNAVVHEFAAACGCGIELSEAALPVKPAVRGVCEL---LGLDALNFANEGKLV 262
SelD        -----FGLLGHLSEMCQGAGVQARVDYEAIPKLPGVEEYIKLGAVPGGTERNFASYGHLM 285

CblS        ------VFNEATMNKTAG-----PASVILVAVKASEVKAFEQNFPAAKCLGELRNYHG-- 247
PurM        QTAGNVEHHEMYRTFNCGVGMIIALPAPEVDKALALLNANGENAWKIGIIKASDSEQRVV 343
ThiL        LCFTVPELNRGALDVALG---HLGVPFTCIGQMTADIEGLCFIRDGEPVTLDWKGYDHFA 323
HypE        IAVERNAAEQVLAALHSH---PLGKDAALIGEVVERKGVRLAGLYGVKRTLDLPHAEPLP 319
SelD        GEMPREVRDLLCDPQTSGG-LLLAVMPEAENEVKATAAEFGIELTAIGELVPARGGRAMV 344

CblS        ---
PurM        IE- 345
ThiL        TP- 325
HypE        RIC 322
SelD        EIR 347
```

Figure S5. Alignment of *Lin*CblS with PurM ATP-binding superfamily proteins. ClustalW2 (Larkin *et al.*, 2007) alignment of *L. innocua* CblS with PurM, ThiL, HypE, and SelD from *E. coli* MG1655. Conserved residues are shaded.

```
A. metaliredigens           -------------------------------------------------M 1
A. oremlandii               --------------------------------------------------
A. colihominis              MCYDNAIEQAAQPPKIDSKEGVPMTMPNQESLWTDYQELLEMTRQCGFSS 50
A. flavithermus             --------------------------------------------------
B. halodurans               --------------------------------------------------
C. hydrogenoformans         -------------------------------------------------M 1
C. botulinum                -------------------------------------------------M 1
C. perfringens              -------------------------------------------------M 1
C. sporogenes               -------------------------------------MYTRYKRKEVSFM 13
C. tetani                   -------------------------------------------------M 1
D. hafniense                --------------------------------------------------
G. kaustophilus             --------------------------------------------------
G. thermodenitrificans      --------------------------------------------------
H. modesticaldum            --------------------------------------------------
L. gravesensis              --------------------------------------------------
L. buchneri                 --------------------------------------------------
L. hilgardii                --------------------------------------------------
L. innocua                  --------------------------------------------------
L. monocytogenes            --------------------------------------------------
L. welshimeri               --------------------------------------------------
L. sphaericus               --------------------------------------------------
M. thermoacetica            --------------------------------------------MTPATL 6
N. thermophilus             --------------------------------------------------
P. acnes                    --------------------------------------MTAPNRVPSRCL 12
S. thermophilum             --------------------------------------------------
T. brockii                  --------------------------------------------------
T. ethanolicus              --------------------------------------------------
T. italicus                 --------------------------------------------------
T. mathranii                --------------------------------------------------
T. pseudethanolicus         --------------------------------------------------
T. tengcongensis            --------------------------------------------------
T. thermosaccharolyticum    --------------------------------------------------

A. metaliredigens           EIKRCRDLTLIERAGQPNLVIACDSCGGIGEKPQDQIKVPAEVVGYFTAR 51
A. oremlandii               -MFKFRDLTVIDIPPNHRMIIACDSSGGIGNKKHDVVQAEPETLGYFTAH 49
A. colihominis              RIRKYRDLSILRLLGDISLVVACDSNASNGEKPNDTHQNSYEETAVSALK 100
A. flavithermus             ----MRDVLCVPLDEENELVLATDCSGGIGLKQDDVVNVPYDVVAYYGAR 46
B. halodurans               --------------------MATDASGGVGEKAKDHVHVPYEVVSYYAAR 30
C. hydrogenoformans         AGFKYRDLTIVPLSRE-KMVIAADSIGGVGPKAGDIVKTSGEIVGRYGAR 50
C. botulinum                NVKKVRDLTLISLDKDKTLVVACDSSGSIGSKKNDILKIPAFYTGKFTIR 51
C. perfringens              QIYKFRDLTVLENEKN-KLVIACDSCGGIGENEGDFVKASNEIVSYFSAR 50
C. sporogenes               NVKKVRDLTLISLDKDKTLVVACDSSGSIGPKKNDILQIPAFYTGKFAIR 63
C. tetani                   RISKVRDLTLIKLTEDKNLVVACDSCGGIGSKPEDALKVPAYIVGKLTAR 51
D. hafniense                MGYQGRDVEVVALNDAQYLVAACDSCGAIGEKELDAVKVPWRVTGRMTAR 50
G. kaustophilus             ----MRDVLFLPFADGMELAVAADGSAAVGDKPGDVVSVPVDVVAYFSAR 46
G. thermodenitrificans      ----MRDVLFLPLTDGVELAIAADGSAAVGEKQGDAVFVPAETTAYFAAR 46
H. modesticaldum            MGYRGRDVEVVALSPEQCLVVACDACGAIGAKELDAVQVSPYIVGIFTTR 50
L. gravesensis              --MKFRDLTIKPISDKTALVIACDVSAGIGEKPDDLVHVTADVTAAFALR 48
L. buchneri                 --MKFRDLTIKPISDKTALVIACDVSAGIGEKPDDLVHVTADVTAAFALR 48
L. hilgardii                --MKFRDLTIKPISDKTALVIACDVSAGIGEKPDDLVHVTADVTAAFALR 48
L. innocua                  -MPQVRDLSVIDVPGG-CVLTSCDISAGFGEKVHDGLRVAPEVTARLTLR 48
L. monocytogenes            -MPQVRDLSVIDVPGG-CVLTSCDISAGFGEKTHDGLMVAPEVTARLTLR 48
L. welshimeri               -MPQVRDLSVVDVPGG-CILTSCDISAGFGEKAHDGLVVTPEVTARLTLR 48
L. sphaericus               -------------MKVGAFIATMDNAAAIGEKPQDIVPASDQLTAYMTAR 37
M. thermoacetica            SPRRYRDLTILDLDAQRSLVIACDSAGAIGPKEADVVRVPGYVLGRFTAR 56
N. thermophilus             MIFQLDDVKMIKMSKNEYLAVACDSLGGIGEKSLDKVKVPCEWVGRVLTR 50
P. acnes                    SITRLRDLLADIPAS-RLVIACDTIGGIGPRPDDSYPADPVWCAHLGAR 61
S. thermophilum             --MRWRDLTLVDLPCGGRLVIACDAAGGIGPKERDVIRVAGYVIGRFTAR 48
T. brockii                  MIERYRDLVIIYEN-DVAYVISCDSLGAIGNKEHDVLKVDEEIVGRTTVK 49
T. ethanolicus              MIERYRDLVVIYEN-DVAYVISCDSLGAIGNKEHDVLKVDEEIVGRTTVK 49
T. italicus                 MIERYRDLVVIYEN-DVAYVISCDSLGAIGNKENDVLKVDEEIVGRTTVK 49
T. mathranii                MIERYRDLVVIYEN-DVAYVISCDSLGAIGNKENDVLKVDEEIVGRTTVK 49
T. pseudethanolicus         MIERYRDLVIIYEN-DVAYVISCDSLGAIGNKEHDVLKVDEEIVGRTTVK 49
T. tengcongensis            MIERYRDLVIIYEK-DVAFVVSCDSVGAIGSKENDILKVDEEIVGRTTLK 49
T. thermosaccharolyticum    MVERYRDVLIIYES-DTVYAIACDSIGAIGNKEGDILKVDEEIVGRTVTK 49

A. metaliredigens           VSLMEVMSVGARVMTVINTLSVEREPTGEKMIKGIQKMIEEVKLP----- 96
A. oremlandii               VALMELLATGATPLTVVNTLGVEMEDSGVRIIEGIQKALEPLNLK---ED 96
A. colihominis              VPLMEVLATGAAPIVIADNLCVEMEPSGRRIIAAMQEELRGCGLYD---- 146
A. flavithermus             VAWMELMSIGATPKAFVLQNFVN-DDAWHALVAGVQQTMEELQLSLP--- 92
B. halodurans               VALLECMSVGATPFTFLVQNFSG-DEPYQKMIDGINRALGEANQAQAT-- 77
C. hydrogenoformans         VVLMELLAVKARPQVISCTVSCEWEPTGKEIYQGVLAEVKTLPFA----- 95
C. botulinum                VGILEVMCTGAEIVTVTVNALCCEMNPTGREIIDGIKGELKRAGID----- 96
C. perfringens              VCLFELLAFRAKPLVIVNNLGMSMNNGGEKIIQGINRAIKEYNAENFFEE 100
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C. sporogenes               VGILEVMCTGAEIVTVTNAVCCEMNPTGKEIIDGIKGELKRAGID----- 108
C. tetani                   VALMEVLCTGAEIVTITDAVCNEMEPTGKEIIRGIKEELKEAKIN----- 96
D. hafniense                VALLEVLAVGAVPQMLSIAIANEPLPAGEEIMKGVREELKAMNLL----- 95
G. kaustophilus             VALMELLSVGAEARVVVLQNFIA-DDRWEALCRGVRRTCRELGIDLP--- 92
G. thermodenitrificans      VALMELVSVGAEAKAVVLQNFIA-DERWEALCRGIRQAGSELGLDLP--- 92
H. modesticaldum            VALMEILATGAEPVALTVTVANEPFPTGGEVIAGVRDELATIGKR----- 95
L. gravesensis              VPLMELLCFGATPISVVDTVGNEMTPTGENMIAGLKQELKRAGLS----- 93
L. buchneri                 VPLMELLCFGATPISVVDTVGNEMTPTGENMIAGLKQELKRAGLS----- 93
L. hilgardii                VPLMELLCFGATPISVVDTVGNEMTPTGENMIAGLKQELRRGGLS----- 93
L. innocua                  VALLEMLASGAVVVAVSDVIGAEMEPTGKRVIAGLKDELFKADLG----- 93
L. monocytogenes            VALIEMIASGAEVVAVSDVVGGEMEPTGRRVIVGLKDELTKANLS----- 93
L. welshimeri               VALLEMIASGADVVSVSDVVGAEMEPTGRRIIIGLKEELSKANLN----- 93
L. sphaericus               VTFLEQLAAQALPIQILLANFSG-DAAWSRYERGIQQVFEETGLPCPV-- 84
M. thermoacetica            VALMEVLALGAWPVCVVNTLCVEPEPAGAAIREGVADEMRVLGIDPEK-- 104
N. thermophilus             VAVMELLALRIDPFLVINTLANEMVPTGEQIISGIKEELDTVEIDP---- 96
P. acnes                    VPLLEVLCAGARPLVLVDTLCQDSASAQPMIAE-FRRCALDAGIDPD--- 107
S. thermophilum             VALMDLLAAGAQPLHLVNNTCVEPDPTGREILQGICDEAALAGLSAD--- 95
T. brockii                  VALSEVLCVGAKPLVISDTLSVEMNPTGQKILRGIKSELEENGLS----- 94
T. ethanolicus              VALSEVLCVGAKPLVISDTLSVEMNPTGQKILRGIKSELEENGLS----- 94
T. italicus                 VALSEVLCVGAKPLVISDTLSVEMNPTGEKILKGIKNELEDNGLL----- 94
T. mathranii                VALSEVLCVGAKPLVISDTLSVEMNPTGEKILKGIKNELEDNGLL----- 94
T. pseudethanolicus         VALSEVLCVGAKPLVISDTLSVEMNPTGQKILRGIKSELEENGLS----- 94
T. tengcongensis            VALSELLSVGATPLVVSDTLSVEMYPTGEKILKGIKKELEENGL------ 93
T. thermosaccharolyticum    VAVSELLCIGAWPIIISDTLSNEMNPTGIKIIGGIKKELNDNEIY----- 94

A. metaliredigens           ----ITALNGSTEENVVTCQTAMGITVIGEVERESIKIGCSK-------- 134
A. oremlandii               -----IVVTGSTEENIPVSQTSMGITIIGMMEKSRWRAQKVE-------- 133
A. colihominis              ----AVSFTGSTEDNMRTLQTGIGVTVIGLVSGASLRLGRTQ-------- 184
A. flavithermus             -------ITGSSESNMPLMQSAVGFVAVGTVRKTEKRINVTP-------- 127
B. halodurans               -------IAGSTESNMVLEQSCLGVTVIGRVHQSALKIGITP-------- 112
C. hydrogenoformans         -----IDITGSSEKNFTTNTTGAGFTAVGLGDELLVNRICET-------- 132
C. botulinum                ----EVVLTGSTEENFPSFSTGLGITVLGIVDNSVIKVNNNVNNFGGNNEK 142
C. perfringens              KSNLSECVTGSTEDNFKTLQTFLGLTIIGEKEET--LKVNFE-------- 140
C. sporogenes               ----EVVLTGSTEENFTTFSTGVGITVLGMVDNSVIKVNSVNNFGNNNEK 154
C. tetani                   ----EVTLNGSTEENFPTKATGLGVTVVGIVDNNNMKVNNIT-------- 134
D. hafniense                ----SLPAAVSTEKNMPTEQTGLGITVIGLCDQDKLRIGRAR-------- 133
G. kaustophilus             -------ITGSTESNFPTVQSALGVTAIGTVANERKRIGITP-------- 127
G. thermodenitrificans      -------ITGSSESNFATVQSALGVTAIGTVAHGQKRIGITP-------- 127
H. modesticaldum            ----DLPLAISTEKNIPTRQTGVGITLVGAVEKERLRVGCSR-------- 133
L. gravesensis              ----DISLNGSTEDNMPTRTTSIGVTVIGIATRRVDDLFQ-K-------- 130
L. buchneri                 ----DISLNGSTEDNMPTQTTSIGVTVIGIATRRVDDLFQ-K-------- 130
L. hilgardii                ----DISLNGSTEDNMPTQTTSIGVTVIGIATRRVDDLFQ-K-------- 130
L. innocua                  ----HIELNGSTEENMNVTQTSVGVLVTGFATKAALKLINVH-------- 131
L. monocytogenes            ----HIELNGSTEENMKVTQTSVGVLVTGFATKAALKLINVH-------- 131
L. welshimeri               ----HIELNGSTEENMKVTQTSVGVLVTGFATKAALKLINVH-------- 131
L. sphaericus               -------IAGSSESNMPTLQSGLAITMLGEIQQ---RKAWDL-------- 116
M. thermoacetica            ------TLTGSSEKNIPTTQSGIGITVIGLATTAELLMGRLA-------- 140
N. thermophilus             ----EISLTGSCETNIVTDQTGAGVTVLGKS-PEFPGWGYSY-------- 133
P. acnes                    ------AVTGSTEDNVATTQTGVGVTIIGVMHHEDVPK--SL-------- 141
S. thermophilum             ------QINGSFEKNIPTVQTGLGVTAIGYLAPGRSLR-TAR-------- 130
T. brockii                  ----DVVFTGSTEENFPTSITGIGITVIAKANVCDLKIKKVK-------- 132
T. ethanolicus              ----DVVFTGSTEENFPTSMTGIGITVIAKANIGDLKIKKVK-------- 132
T. italicus                 ----DVVFTGSTEENFPTSMTGIGITVIAKAYVCDLKIKKVK-------- 132
T. mathranii                ----DVVFTGSTEENFPTSMTGIGITVIAKANVCDLKIKKVK-------- 132
T. pseudethanolicus         ----DVVFTGSTEENFPTSITGIGITVIAKANVCDLKIKKVK-------- 132
T. tengcongensis            ----EVVLTGSTEENFPTSMTGIGITAVGRARKEDLKIKKAR-------- 131
T. thermosaccharolyticum    ----DVALTGSTEENFPSTMTGVGVTAIGKAEKEALKVRKAM-------- 132

A. metaliredigens           ------PGDLIVALGTPKVGNEIK--LPI-DDEICSIKDFQALVKMKNVK 175
A. oremlandii               ------KGDLAVVLGIPKVGQEVLEDEGR---EIMSIPLLLELLKKKSIH 174
A. colihominis              ------RGDAVYCAGVPQSGVLERYSEHDP--SVAKISTVTRLCALDYIH 226
A. flavithermus             ------TDACWAVIGEPLVGEAVIQKKDR----IIPLSLFRTLLQLDGIY 167
B. halodurans               ------KDAGIALLGKPLVGEEVVSQEKD----VFPLKQFDELLQEEGVY 152
C. hydrogenoformans         --------SDLYIVGVPLVGEEVLKFPELQ----ATPHLVYELSQNNDVL 170
C. botulinum                HNK---DDILLISVGIPKVGEEIN--IYD-DKEIVDYEDIKILLENPKVY 186
C. perfringens              ------KGDSICLLGIPKVGQEVLEDINNNLGEIVTFKDFKILMDNKDVK 184
C. sporogenes               HNKFDEDDVLLISVGIPKLGKEIN--IYD-DKEIVDYVDIKILLENPRVY 201
C. tetani                   ------KDCLLISIGIPKVGKEID--IFGYDEEIASYNNISTILNNQEAY 176
D. hafniense                ------PGNSLFCLGLPKVGTEAADPEDP---DILQGIHLIQLLNIPGVY 174
G. kaustophilus             ------EEAKFAVIGRPLVGSAVLSHPEW----VAPLPLFAALLRTPYIY 167
G. thermodenitrificans      ------ETAKFAVIGRPLVGSAVLVHSDW----IAPLSLVAELLASPYVY 167
H. modesticaldum            ------PGDCVYSLGWPKVGAEVVVDDGK---AIARADHVQALLAHPGVH 174
L. gravesensis              ------QPMVIYQLGRPLVG--DAVKQHFS--DLFSYNLINELRSDSAVI 170
L. buchneri                 ------QPMVIYQLGRPLVG--YAVKQHFN--DLFSYNLINELRSDSAVI 170
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L. hilgardii                 ------QPMVIYQLGRPLVG--YAVKQHFN--DLFSYNLINELRSDSAVI 170
L. innocua                   ------EAAVLFAFGEPIVG--AEVLQRMM--EMPDYPLVKQLVSDSRVL 171
L. monocytogenes             ------EAAVLFAFGEPIVG--AEVLQKLR--DMPDYRLVKKLVEHNDVL 171
L. welshimeri                ------EAAVLFAFGEPIVG--EEVLQKMT--EMPDYHLVKKLVADSSVL 171
L. sphaericus                ------EQLSWYTYGLPLVGEELLAQPDD----VAQLQPIYQAWLAEIVQ 156
M. thermoacetica             ------AGDTLALFGRPKVG--TEVFLDD--PEIVDLKTVRLLLDQPGIR 180
N. thermophilus              ------PGIALYMIGTPKVGAEVDLND-P---EIMDLQTMKELSEINGVL 173
P. acnes                     ------DGDVLVCVGAPISAPNDDVALGR--REIVGVTEVKALMASGKVH 183
S. thermophilum              ------PGDLVVAIGRPKVG--AEVRLDD--PELPDLPLVRRLAGDPLVH 170
T. brockii                   ------AGMHVSLLGYPRVGSEVLGAK-----DVLNLSDYIKISNSKEIV 171
T. ethanolicus               ------TGMHVSILGYPRVGNEVLSSN-----DVLTLKDYVKISNSKEIV 171
T. italicus                  ------AGMHVSLLGYPRVGSEVLSSK-----DVLTLKDYIKISNSKEII 171
T. mathranii                 ------AGMHVSLLGYPRVGSEVLNSK-----DVLTLKDYIKISNSKEII 171
T. pseudethanolicus          ------AGMHVSLLGYPRVGSEVLGAK-----DVLNLSDYIKISNSKEIV 171
T. tengcongensis             ------KGMHVVLIGYPRVGSEVLGAE-----DVMTLKDYIKISQTKEVV 170
T. thermosaccharolyticum     ------AGMEVGLFGYPRVGQEVLCCH-----DILSLKDYVKIFRCGEIV 171

A. metaliredigens            DIHPIGSKGMYYEAQLLASLNHCEFKSREA-TGVD--LKKSAGPATAVIF 222
A. oremlandii                DILPVGSKGIAYEVGQMAESNGIGYNLY-EHVGID--LNKSAGPATCVIV 221
A. colihominis               EILPVGSKGAAYEAGQLAECVGCTFAAD-AQPPID--LATSAGSCTAVLV 273
A. flavithermus              EIIPVGSKGISHEWKLMYGE-----RPLRCS--LP--PHASAGPATCVLV 208
B. halodurans                EIIPVGSKGVKYELEVLAATNEWNTLQVPVNGNVD--LTKSSGPATSLLI 200
C. hydrogenoformans          EIIPCGSGGVGAELMWLKNQG---FEIENVIAPPFS-LEKSAGPGTSLLV 216
C. botulinum                 EIVPVGSKGILYEGEILARNNNLKLKLEEN-ISID--IKKSNGPATTIIA 233
C. perfringens               DILPIGSKGIIHEISELENSDKIRINL--SYEGDY--LRKSAGPATALLF 230
C. sporogenes                EIVPVGSKGILYEGEVLAKNNNLKLKLEEN-IPID--IKRSNGPATTVIA 248
C. tetani                    EIVPVGSKGILFEAEELAKNNRCEFYLNKDKIDVD--IRRSAGPATVIIA 224
D. hafniense                 DIIPVGSQGIRGEAEALAQAVGARFGE-DSQCRLD--INKSAGPSTCLIF 221
G. kaustophilus              ELIPIGSKGIYYEWTQLLAANGRQGRACACP--LP--LFSSGGPATSVLV 213
G. thermodenitrificans       ELIPIGSKGIYYEWTQLLAANGRQWCACACP--LP--LFASGGPATSLLI 213
H. modesticaldum             EVLPVGSKGIRAEAEKLAAAVHCRICEWQSVAGLD--LDKSAGPSTCLVF 222
L. gravesensis               DMLPVGSKGIAFEINQLAKTHQLKVVDGSVMETEE--MVKSAGPATVALI 218
L. buchneri                  DMLPVGSKGIAFEINQLAKTHQLEIVDSSVMETEE--MAKSAGPATVALI 218
L. hilgardii                 DMLPVGSKGIAFEINQLAKTHQLEIVDSSVMETEE--MAKSAGPATVALI 218
L. innocua                   EVVPVGSKGMAYEANTLARLNDCVFEASGVFNEAT--MNKTAGPASVILV 219
L. monocytogenes             EVVPVGSKGMAYEAHLLARLNGGIFVPSDTFSEAV--MNKTAGPASVILA 219
L. welshimeri                EVVPVGSKGILYEANLLAKLNNRVFKETSSLDEIS--LNKTAGPASVILT 219
L. sphaericus                QVWPVGSKGLQGECARLFGQ-----QLVECS--HD--MTKSAGPSTVILL 197
M. thermoacetica             EIVPAGSRGILAEARDLAALYGLQINWRPGLTGPA--LLKSAGPATCILA 228
N. thermophilus              EILPVGSQGLLKESNTLANRSELRLQLNNAIKTDEGLLTKSAGPSTCVIF 223
P. acnes                     DCVPVGSHGVAWEAKQLASTAGLRVAFQP--TDVD--LTRSGGPATCVVM 229
S. thermophilum              DLLPVGSRGIRAEAEDLAASAGLEVEWAPAEAGFP--LGKSAGPVTCLLV 218
T. brockii                   EAIPVGSKGVKYEIGILEKISGLKVEA-NFPQHLD--VLKSGGPSTCCLV 218
T. ethanolicus               EAIPVGSKGVKYEIGILEKISGLKVEA-NFPQHLD--VLKSGGPSTCCLV 218
T. italicus                  EAIPVGSKGIKYEIGILEKISGLKVEA-NFPQYLD--VLKSGGPSTCCLV 218
T. mathranii                 EAIPVGSKGIKYEIGILEKISGLKVEA-NFPQYLD--VLKSGGPSTCCLV 218
T. pseudethanolicus          EAIPVGSKGVKYEIGILEKISGLKVEA-NFPQHLD--VLKSGGPSTCCLV 218
T. tengcongensis             EAIPVGSKGIAYELGVLEDLYGFRIKE-DDGLNID--LFKSAGPATCCLV 217
T. thermosaccharolyticum     EAIPVGSKGIKHELDVLKLSSGLEFLK-EYKSDLD--DTKSGGPSTCCIV 218

A. metaliredigens            SVSKE-QLPT--VESQLQQQVKVIGSLEN------------ 248
A. oremlandii                AVNQE-NYEA--LKESMPIPVHLVGSFT------------- 246
A. colihominis               SLPLQ---AGGRLAADMDVPCFLIGRIQ------------- 298
A. flavithermus              SYHQK---QEPLLQSLAGTLFYPIVNEL------------- 233
B. halodurans                TFDGT---MEALLKAKYSSWWTTLASKIST----------- 227
C. hydrogenoformans          AVKEG-------IELKAAIPVHYLGKAIKVKKT-------- 242
C. botulinum                 AIHKE-EYEN--IRAKINN-VNIIGKLER------------ 258
C. perfringens               ILRNN-SLED--IKNKIKTPIMEIGNII------------- 255
C. sporogenes                AIHKE-EYEN--IRAKINN-VNIIGELESV----------- 274
C. tetani                    AVSKK-AYEN--LKD-IQN-INLLGEIKKGT---------- 250
D. hafniense                 TAAED-----IELGDFGKLPIHKIGRLEEDGGGGLLTENED 257
G. kaustophilus              SYDPD---GEREIKKQAGSLFFPLHVEW------------- 238
G. thermodenitrificans       SYDPA---GEQVLRKQAGRLFFSLFAEL------------- 238
H. modesticaldum             TAGP-------KPPEIAGAPIHYLGRLSESAG--------- 247
L. gravesensis               GVKPE---KRRVFERHFPQVSYLMSLT-------------- 242
L. buchneri                  GVKPE---KRRVFERHFPQAHYLLSLN-------------- 242
L. hilgardii                 GVKPE---KRRVFERHFPQAHYLLSLN-------------- 242
L. innocua                   AVKAS---EVKAFEQNFPAAKCLGELRNYHG---------- 247
L. monocytogenes             AVKED---NVTAFEREFLGAKRIGLLRGNEEWT-------- 249
L. welshimeri                AVKKR---DCSIFEKHFSGAQYLGELCGNEEQI-------- 249
L. sphaericus                GMDPE---KEQLAHQFFQRNFEKLRISAE------------ 223
M. thermoacetica             AGDRA-ALETTGRRA--EKPFCLLGTLGPAASR-------- 258
N. thermophilus              TADHDGADQAQKLAQVSGKKMTQLGRLY------------- 251
P. acnes                     ACAKT-NLEQLHALVNPERPWAVIGHLRS------------ 257
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S. thermophilum            AAAPS-ALQGLALTL--TQPWAVVAQLR------------- 243
T. brockii                 VHNEE---DTVSIKGLTDKPLTYVGVLI------------- 243
T. ethanolicus             VHSEE---DTASIKGLTDKPLTYVGVLI------------- 243
T. italicus                VHNEE---DTVSIKGLTDKPLTYIGVLI------------- 243
T. mathranii               VHSEE---DTVSIKGLTDKPLTYIGVLI------------- 243
T. pseudethanolicus        VHNEE---DTVSIKGLTDKPLTYVGVLI------------- 243
T. tengcongensis           VYREE---DTDFIKAITDKPFTHVGIIE------------- 242
T. thermosaccharolyticum   VYKEG---DRRKIENLLDKPFVRLGRLIDGR---------- 246
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Figure S6. Alignment of CblS homologs. ClustalW2 (Larkin *et al.*, 2007) alignment of predicted CblS proteins from 32 bacterial species (Table S5). Conserved residues are shaded.

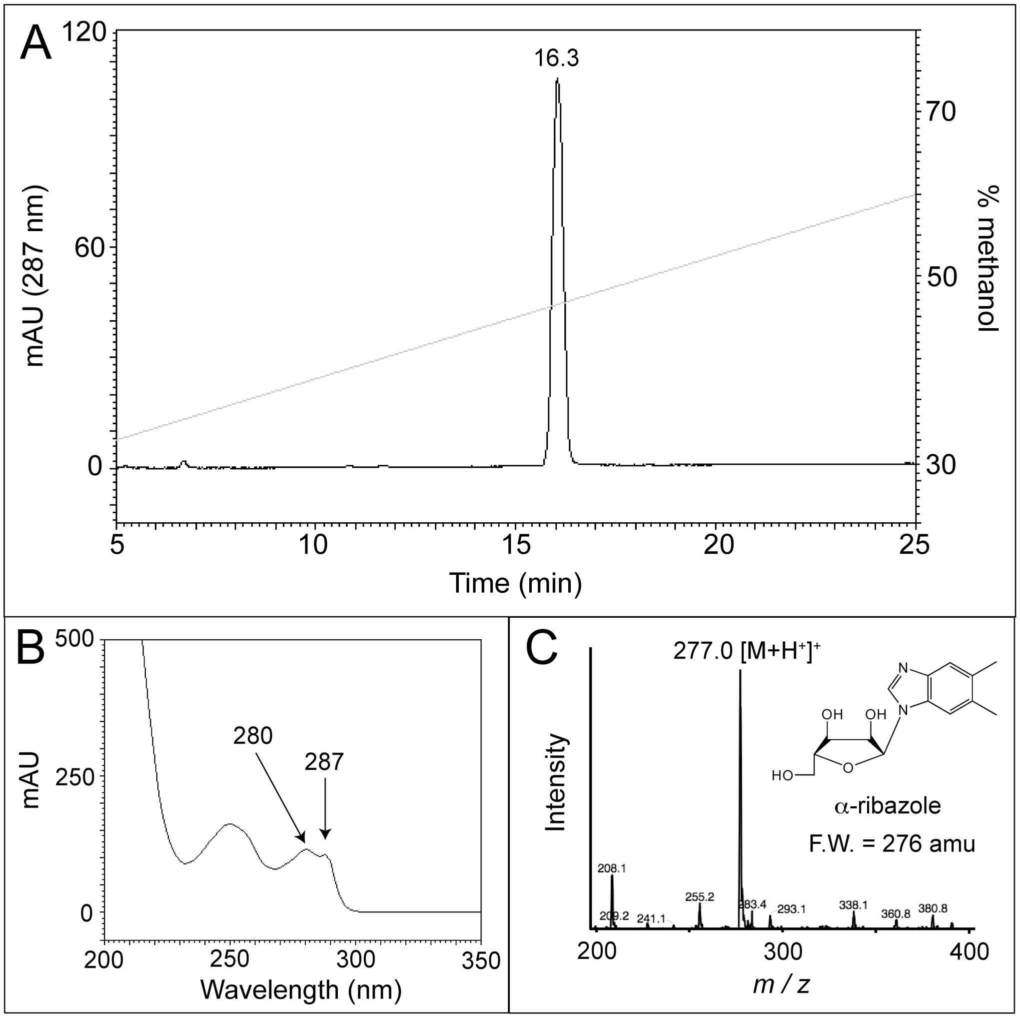

Figure S7. Purification of α-R. Purified α-R was resolved by RP-HPLC using a Beckman Coulter System Gold® 126 HPLC system equipped with a Phenomenex 150 x 4.6 mm Synergi 4μ Hydro-RP column. Products were detected by their absorbance at 287 nm using a photodiode array detector. The column was equilibrated at 1 ml min^{-1} with 30% methanol. 5 minutes after injection, the column was developed for 20 min with a linear gradient to 60% methanol, then developed for 5 min with a linear gradient to 100% methanol. Panel A shows elution of a product at 16 min. The grey line (right axis) indicates the methanol gradient used to elute α-R. Panels B and C show the UV-visible absorbance and mass spectra of α-R, respectively. The structure of α-R is indicated in the inset to panel C.

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