SUPPLEMENTAL INFORMATION

Genetic and biochemical characterizations of enzymes involved in *Streptococcus pneumoniae* serotype 2 capsule synthesis demonstrate that Cps2T (WchF) catalyzes the committed step by addition of β1-4 rhamnose, the second sugar residue in the repeat unit

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Running title: Cps2T (WchF) rhamnosyltransferase activity

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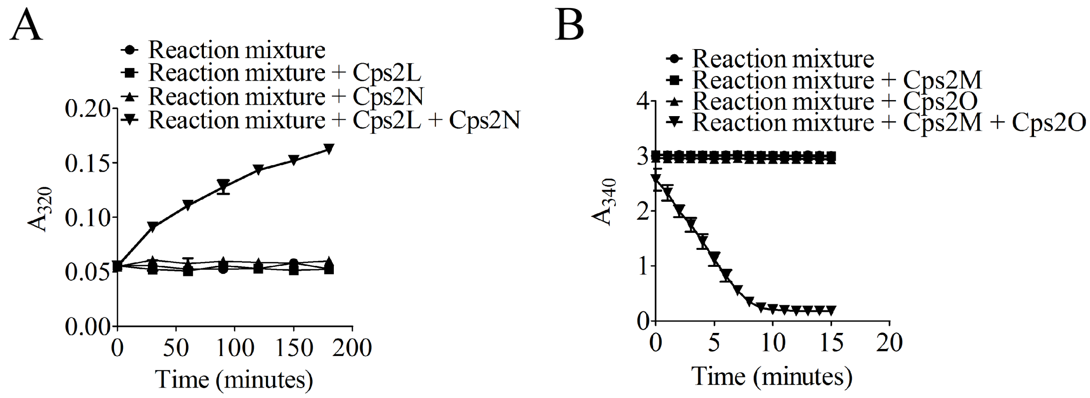

FIG S1. Kinetic assays for Cps2N and Cps2O. Assays were performed as described in the Materials and Methods. The reaction mixture for Cps2N activity (A) contained dTTP, Glc-1-P, NAD^+, and the indicated enzyme(s). Activity was measured as the generation of NADH. The reaction mixture for Cps2O activity (B) contained the Cps2N product, NADH, and the indicated enzyme(s). Activity was measured as the oxidation of NADH. Data points represent means ± standard errors from three reactions.

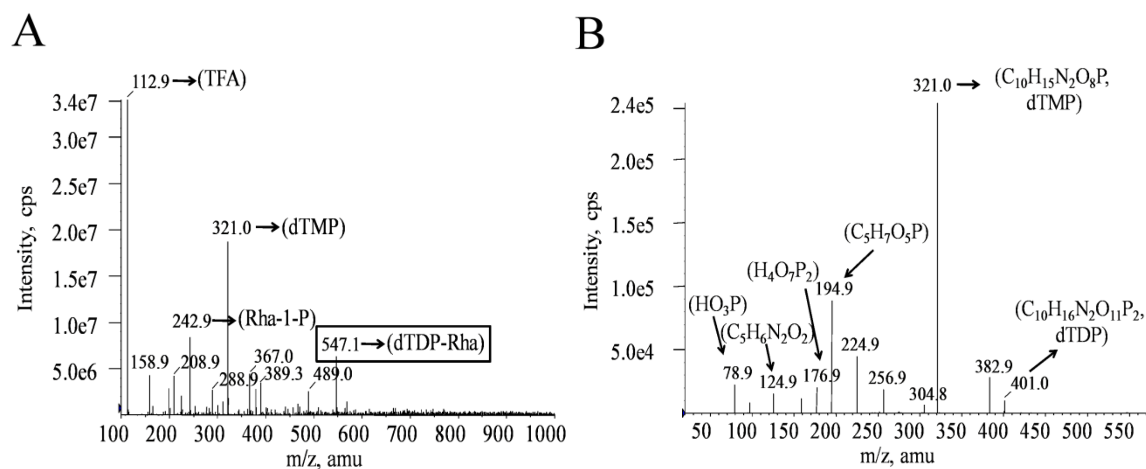

FIG S2. ESI-MS/MS of HPLC purified Cps2O product. One µl of purified dTDP-Rha (approximately 1 mM) was injected by direct infusion into a triple quadruple mass spectrometer operated under the negative electrospray ionization mode. (A) Precursor ion spectra of the purified Cps2O product (ESI-MS). Ion 547.1 corresponds to dTDP-Rha . (B) The product ion spectra (ESI-MS/MS) of ion 547.1. Product ions indicated are consistent with calculated fragments of dTDP-Rha. Ion intensity is presented as counts per second (cps).

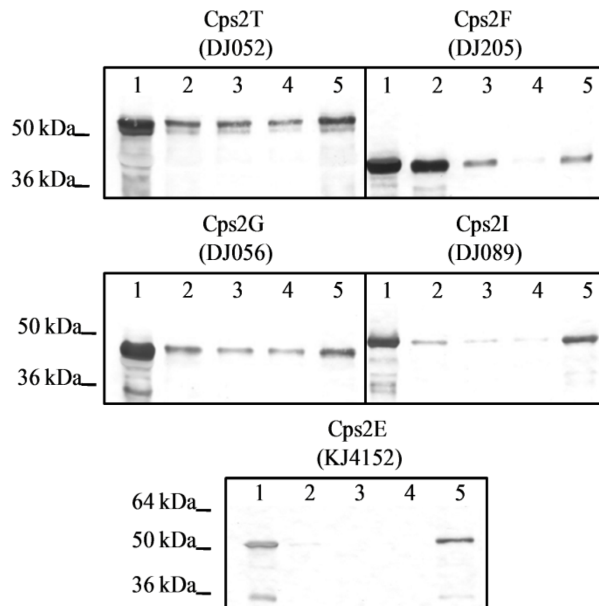

FIG S3. Cps2E, Cps2T, Cps2F, Cps2G, and Cps2I membrane association in *E. coli*. Membrane fractions (3 µg of total protein) were sequentially washed with 30 µl of 100 mM Na_2CO_3 (pH 11.5). Lanes: 1, 3 µg of total membrane protein from the indicated strains; 2, first wash with 100 mM Na_2CO_3 (the entire 30 µl was loaded into the gel); 3 and 4, second and third washes with 100 mM Na_2CO_3, respectively; 5, remaining proteins associated with the membrane. Cps2T, Cps2F, Cps2G, and Cps2I blots were probed with α-Tetra-His; the Cps2E blot was probed with α-Cps2E.

TABLE S1. Additional strains and plasmids used in this study

Strain(s) or plasmid(s)	Properties[a]	References or source
E. coli strains		
BL21-AI	F^- *ompT hsdSB*(r_B^- m_B^-) *gal dcm araB::*T7 RNAP-*tetA*	Invitrogen
DB11	*met thi gal hasdR nal rif*	(3)
DJ005	BL21-AI (pDJ005); full length Cps2I; Ap^R	This study
DJ009	BL21-AI (pDJ009); full length Cps2T; Ap^R	This study
DJ011	BL21-AI (pDJ011); full length Cps2G; Ap^R	This study
DJ014	BL21-AI (pDJ014); full length Cps2M with N-terminal-His_6; Ap^R	This study
DJ015	BL21-AI (pDJ015); full length Cps2N with N-terminal-His_6; Ap^R	This study
DJ016	BL21-AI (pDJ016); full length Cps2O with N-terminal-His_6; Ap^R	This study
DJ017	BL21-AI (pDJ017); full length Cps2L with N-terminal-His_6; Ap^R	This study
DJ052	BL21-AI (pDJ052); full length Cps2T with N-terminal-His_6; Ap^R	This study
DJ056	BL21-AI (pDJ056); full length Cps2G with N-terminal-His_6; Ap^R	This study
DJ078	DB11(pDJ078); *cps2T* deletion construct; Em^R	This study
DJ086	DB11(pDJ086); *cps2F* deletion construct; Em^R	This study
DJ089	BL21-AI (pDJ089); full length Cps2I with N-terminal-His_6; Ap^R	This study
DJ131	DB11(pDJ131); *cps2G* deletion construct; Em^R	This study
DJ184	DB11(pDJ184); *cps2I* deletion construct; Em^R	This study
DJ192	DB11(pDJ192); Δ*cps2T* repair construct; Em^R	This study
DJ204	BL21-AI (pDJ204); full length Cps2F, replaced GTG start with ATG; Ap^R	This study
DJ205	BL21-AI (pDJ205); full length Cps2F, replaced GTG start with ATG, N-terminal-His_6; Ap^R	This study
KJ4152	BL21-AI (pKJ4152); full length Cps2E; Ap^R	This study
RC124	BL21-AI (pET-20b); Ap^R	(1)
Plasmids		
pCR TOPO 2.1	PCR cloning vector; Ap^R Km^R	Invitrogen
pDL276	*aphA-3* containing vector; Km^R	(2)
pDJ005	pET-20b/*cps2I* amplified from D39 using primers I7/I8	This study
pDJ009	pET-20b/*cps2T* amplified from D39 using primers T13/T14	This study
pDJ011	pET-20b/*cps2G* amplified from D39 using primers G10/G11	This study
pDJ014	pET-16b/*cps2M* amplified from D39 using primers M4/M5	This study
pDJ015	pET-16b/*cps2N* amplified from D39 using primers N4/N5	This study
pDJ016	pET-16b/*cps2O* amplified from D39 using primers O3/O4	This study
pDJ017	pET-16b/*cps2L* amplified from D39 using primers L6/L7	This study
pDJ052	pET-16b/*cps2T* amplified from D39 using primers T13/T14	This study
pDJ056	pET-16b/*cps2G* amplified from D39 using primers G10/G11	This study
pDJ078	pJY4164 containing *cps2E* (amplified from D39 using primers E54/E55), *aphA-3* (amplified from pSF151 using primers DJ-01/DJ-02), and *cps2F* (amplified from D39 using primer T12/F12). Used for *cps2T* deletion	This study
pDJ086	pJY4164 containing *cps2T* (amplified from D39 using primers T10/T11), *aphA-3* (amplified from pSF151 using primers DJ-01/DJ-02), and *cps2G* (amplified from D39 using primers F7/G8). Used for *cps2F* deletion	This study
pDJ089	pET-16b/*cps2I* amplified from D39 using primers I6/J8	This study
pDJ131	pJY4164 containing *cps2F* (amplified from D39 using primers F8/F9), *aphA-3* (amplified from pSF151 using primers DJ-01/DJ-02), and *cps2H* (amplified from D39 chromosomal DNA using primer G9/H7). Used for *cps2G* deletion.	This study
pDJ184	pJY4164 containing *cps2H* (amplified from D39 using primers H8/F9), *aphA-3* (amplified from pSF151 using primers DJ-01/DJ-02), and *cps2J* (amplified from D39 chromosomal DNA using primer I9/J9). Used for *cps2I* deletion.	This study
pDJ192	pJY4164/*cps2ETF* amplified from D39 using primers E8/F12. Used to repair Δ*cps2T* mutants.	This study
pDJ204	pET-20b/*cps2F* amplified from D39 using primers F10/F12. GTG start codon changed to ATG.	This study
pDJ205	pET-16b/*cps2F* amplified from D39 using primers F10/F12. GTG start codon changed to ATG.	This study
pET16b	Protein expression vector, Ap^R	Novagen
pET20b	Protein expression vector, Ap^R	Novagen
pJY4164	*S. pneumoniae* suicide vector; Em^R	(5)
pKJ4152	pET-20b/*cps2E* amplified from D39 using primers E8/E11.	This study
pSF151	Streptococcal shuttle vector, derivative of pDL276	(4)

[a]Km^R, kanamycin resistant; Ap^R, ampicillin resistant; Em^R, erythromycin resistant.

TABLE S2. Primers used in this study

Primer[a]	Sequence[b]	Description[c]
E8 (+)	*catATG*AATGGAAAAACAGTAAAGTC	*cps2E*$^{5046–5068}$
E11 (-)	*ctcgag*CTACTTCGCTCCATCTCTC	*cps2E*$^{6414–6395}$
E54 (+)	gcgc*ggtacc*ATGAATGGAAAAACAGTAAAGTCTTC	*cps2E*$^{5046-5071}$
E55 (-)	gcgc*gcggccgc*CTACTTCGCTCCATCTCTCATAAATAC	*cps2E*$^{6413-6384}$
F7 (+)	gcgc*ctcgag*GTGTGGTGCAAGTAATCGGTG	*cps2F*$^{8529-8549}$
F8 (+)	gcgc*ggtacc*gTGAGTAACAAGCAAATTGCGATTATG	*cps2F*$^{7622-7648}$
F9 (-)	gcgc*gcggccgc*CTAAATAAACATTAACTCACCGATTACTTGC	*cps2F*$^{8566-8536}$
F10 (+)	*cataTG*AGTAACAAGCAAATTGCGATT	*cps2F*$^{7623-7648}$
F12 (-)	gcgc*ggatcc*CTAAATAAACATTAACTCACCGATTACTTGC	*cps2F*$^{8566-8536}$
G8 (-)	gcgc*ggatcc*CTATTTACCGTTTTCAATATATACCCC	*cps2G*$^{9628-9602}$
G9 (+)	gcgc*ctcgag*GGGGTATATATTGAAAACGGTAAATAG	*cps2G*$^{9602-9628}$
G10 (+)	*catATG*AAAATTAATTTTATCCTTCCATTTAAG	*cps2G*$^{8582-8611}$
G11 (-)	*gaattc*CTATTTACCGTTTTCAATATATACCCC	*cps2G*$^{9628-9602}$
H7 (-)	gcgc*ggatcc*TTATTTTTCTTGCTTAGTCAATCTCATTC	*cps2H*$^{10832-10804}$
H8 (-)	gcgc*gcggccgc*TTATTTTTCTTGCTTAGTCAATCTCATTC	*cps2H*$^{10832-10804}$
H9 (+)	gcgc*ggtacc*ATGCTCTCTCTATATACAGGAAATGGTG	*cps2H*$^{9669-9696}$
I6 (+)	*ctcgag*ATGACAAAAAGTATCTTATATTTTTTATCTACATC	*cps2I*$^{10962-10985}$
I7 (+)	*catATG*ACAAAAAGTATCTTATATTTTTTATCTACATC	*cps2I*$^{10962-10996}$
I8 (-)	*gaattc*TCAATTTTCTAGTTCCTTATATAGTTGCATG	*cps2I*$^{12119-12089}$
I9 (+)	gcgc*ctcgag*GATAAAAATAATGCCATGCAGATCATG	*cps2I*$^{12066-12083}$
J8 (-)	*ggatcc*TCAATTTTCTAGTTCCTTATATAGTTGCATG	*cps2J*$^{12117-12088}$
J9 (-)	gcgc*ggatcc*TTATGTTAGAAACTTTTTTAATTCACCAATAATAC	*cps2J*$^{13525-13491}$
L6 (+)	*catATG*AAAGGTATTATTCTTGCGGGTG	*cps2L*$^{15510-15534}$
L7 (-)	*gctaagc*CTAGACTTCTCCAATCAAACGGAGC	*cps2L*$^{16389-16359}$
M4 (+)	*catATG*ACAGATAATTTTTTCGGAAAAATAC	*cps2M*$^{16380-16407}$
M5 (-)	*ggatcc*TTACAAATCTTCTTTTTTCAAAGGTTTTAC	*cps2M* $^{16973-16944}$
N4 (+)	*catATG*ACTGAATACAAAAATATTATCGTGAC	*cps2N*$^{16985-17014}$
N5 (-)	*ggatcc*TTATACTGTAATAATCTCCTGAGTCTTAGC	*cps2N*$^{18035-18006}$
O3 (-)	*ctcgag*TTATCTCACTTCTTGTTTGTAAAATTCTTG	*cps2O*$^{18952-18923}$
O4 (+)	*catATG*ATTTTAATTACAGGGGCAAATG	*cps2O*$^{18101-18125}$
T10 (+)	gcgc*ggtacc*ATGAAGAAGTCAGTTTATATCATTGGTTC	*cps2T*$^{6445-6473}$
T11 (-)	gcgc*gcggccgc*TTACTCACTTTTTCCCCCTTCAAAC	*cps2T*$^{7629-7605}$
T12 (+)	gcgc*ctcgag*GAAGAGATAGTGGTGGATTATGAGGAAG	*cps2T*$^{7576-7603}$
T13 (+)	*catATG*AAGAAGTCAGTTTATATCATTGGTTC	*cps2T*$^{6445-6473}$
T14 (-)	*ctcgag*TTACTCACTTTTTCCCCCTTCAAAC	*cps2T*$^{7629-7605}$
DJ-01 (+)	gcgc*gcggccgc*GAGGAAGGAAATAATAA	*aphA-3*$^{1729-1746}$
DJ-02 (-)	gcgc*ctcgag*GTACTAAAACAATTCATCCA	*aphA-3*$^{2543-2523}$

[a] Forward and reverse primers are indicated by + and – , respectively.

[b] Lower case letters indicate restriction enzyme sites inserted for digestion. Restriction enzyme sites are italicized.

[c] Superscripts indicate the start and end positions of the primers in the type 2 D39 capsule locus. (GenBank accession no. AF026471) and pDL276 *aphA-3* (GenBank accession no. AF216803)

TABLE S3. Frequencies of acquiring deletion mutations

Recipients were the D39 parent and the D39 derivative BX515, which contains a *cps2E* point mutation

		Transformant Frequency[a]	
Mutation	Exp #[b]	D39	BX515
Δ*cps2T* [c]	1	290/116,000 (2.5 x 10^{-3})	410/25,000 (1.6 x 10^{-2})
	2	710/34,000 (2.1 x 10^{-2})	380/48,000 (7.9 x 10^{-3})
	mean	1.2 x 10^{-2} (9.2 x 10^{-3})	1.2 x 10^{-2} (4.2 x 10^{-3})
Δ*cps2F* [c]	1	280/92,000 (3.0 x 10^{-3})	74/27,000 (2.7 x 10^{-3})
	2	270/51,000 (5.3 x 10^{-3})	64/22,000 (2.9 x 10^{-3})
	mean	4.2 x 10^{-3} (1.1 x 10^{-3})	2.8 x 10^{-3} (8.4 x 10^{-5})
Δ*cps2G* [c]	1	3/90,000 (3.3 x 10^{-5})	54/26,000 (2.1 x 10^{-3})
	2	2/64,000 (3.1 x 10^{-5})	79/25,000 (3.2 x 10^{-3})
	mean	3.2 x 10^{-5} (1.0 x 10^{-6})	2.6 x 10^{-3} (5.4 x 10^{-4})[d]
Δ*cps2I* [c]	1	4/87,000 (4.6 x 10^{-5})	7/25,000 (2.8 x 10^{-4})
	2	1/88,000 (1.1 x 10^{-5})	11/32,000 (3.4 x 10^{-4})
	mean	2.9 x 10^{-5} (1.7 x 10^{-5})	3.1 x 10^{-4} (3.2 x 10^{-5})[d]

[a] within each mutation group, data above the line are the number of antibiotic resistant CFU/total CFU for two independent experiments, with the calculated frequencies shown in parentheses. The data below the lines are the means of the frequencies from the two experiments, with ± standard errors shown in parentheses.

[b] experiment number for two independent experiments using different batches of competent *S. pneumoniae* and different preparations of donor DNA.

[c] donor DNAs used for deletions were obtained from *E. coli* constructs indicated in Table S1.

[d] significantly different ($P<0.05$) from D39, as determined using a two-tailed unpaired Student *t*-test.

SUPPLEMENTAL REFERENCES

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