Supplementary Table S1. Gene contents of the two large unique regions in *L. reuteri* JCM 1112^T compared to *L. reuteri* DSM 20016^T

Start	End	Direction	Gene	Product Name
442995	**451429**			**Unique region I**
442840	444414	-	LAR_0380	transposase
445084	446091	+	LAR_0381	glyceraldehyde 3-phosphate dehydrogenase
446182	447387	+	LAR_0382	phosphoglycerate kinase
447503	448252	+	LAR_0383	triosephosphate isomerase
448292	449665	+	LAR_0384	enolase
449836	451446	+	LAR_0385	transposase
1064161	**1094397**			**Unique region II**
1065608	1066297	-	LAR_0936	nitrate reductase gamma subunit
1066290	1066868	-	LAR_0937	nitrate reductase delta subunit
1066861	1068420	-	LAR_0938	nitrate reductase beta subunit
1068410	1072075	-	LAR_0939	nitrate reductase alpha subunit
1072221	1073240	+	LAR_0940	molybdopterin biosynthesis protein MoeB
1073237	1073734	+	LAR_0941	molybdopterin biosynthesis protein MoaB
1073724	1074941	+	LAR_0942	molybdopterin biosynthesis protein MoeA
1074920	1075408	+	LAR_0943	molybdopterin-guanine dinucleotide biosynthesis protein MobB
1075408	1075980	+	LAR_0944	molybdopterin-guanine dinucleotide biosynthesis protein MobA
1075970	1076254	+	LAR_0945	conserved hypothetical protein
1076405	1077460	+	LAR_0946	two component sensor histidine kinase
1077453	1078106	+	LAR_0947	two component response regulator
1078202	1078474	+	LAR_0948	conserved hypothetical protein
1078480	1079448	+	LAR_0949	conserved hypothetical protein
1079453	1080358	+	LAR_0950	conserved hypothetical protein
1080419	1081612	-	LAR_0951	nitrite extrusion protein
1081659	1081904	-	LAR_0952	molybdopterin biosynthesis protein MoaD
1081904	1082308	-	LAR_0953	molybdopterin biosynthesis protein MoaE
1083097	1083873	+	LAR_0954	iron(III) ABC transporter permease component
1083870	1084646	+	LAR_0955	iron(III) ABC transporter ATP-binding component
1084700	1085722	-	LAR_0956	iron(III) ABC transporter substrate binding component
1088145	1089527	-	LAR_0957	reverse transcriptase/maturase family protein
1090122	1093811	-	LAR_0958	conserved hypothetical protein

Supplementary Table S2. Glycerol dehydratase activity in *E. coli* containing DNA cloned from *L. reuteri* JCM 1112^T

Cell extract	Insert DNA[a]	Recipient	Medium	Glycerol dehydratase activity (units / wet g)[b]
Control	No insert	BL21 (DE3) RIL	LB broth	< 0.1
Clone 1	*gupCDE* of JCM 1112	BL21 (DE3) RIL	LB broth	4.97±1.11
Clone 2	*gupC* of JCM 1112	BL21 (DE3) RIL	LB broth	< 0.1
Clone 3	*gupD* of JCM 1112	BL21 (DE3) RIL	LB broth	< 0.1
Clone 4	*gupE* of JCM 1112	BL21 (DE3) RIL	LB broth	< 0.1
L. reuteri	-	-	MRS broth	250

a) The recombinant plasmid was constructed by cloning the fragments amplified by PCR from the JCM 1112^T genomic DNA into a vector pET101/D-TOPO (Invitrogen Co.), and the insert was confirmed by DNA sequencing. The cloning step was performed in Chemically Competent *E. coli* TOP10 (Invitrogen Co.) and the expression experiments were conducted in *E. coli* BL21-CodonPlus (DE3)-RIL Competent Cells (Stratagene Co.) in the presence of isopropyl 1-thio-b-D-galactoside (IPTG). The primer sequences were as follows: forward (5'-CACCATGAAACGTCAAAAACGATTT-3'); reverse (5'-ATTAAATCACCTGTTTGCCATTTCC-3').

b) The cell extracts were prepared in a total volume of 1.0 ml solution containing 0.2 M glycerol 0.05 M KCl, 0.035 M phosphor-potassium buffer (pH 8.0) and 15 μM adenosylcobalamine. The dehydratase activity was measured at 37°C for 10 min by the 3-methyl-2-benzothiazolinonehydrazone (MBTH) method[S1]. The activity of all clones was obtained from the same experiments performed three times. All experiments were performed using the DNA cloned in pET101 except the experiment for JCM 1112^T.

Supplementary Table S3. Growth of *Lactobacillus delbrueckii* subsp. *lactis* ATCC 7830 in vitamin B_{12}-free medium

Supplements	Incubation time at 37°C (O.D.560)	
	0 hr	10 hr
Cell extract of *L. reuteri* JCM 1112^T	0.03	1.35
Cell extract of *L. plantarum* WCFS1	0.03	0.09
Saline (0.75% NaCl)	0.02	0.08
Cyanocobalemin (0.5 μg/ml)	0.02	1.07
Cyanocobalemin (0.7 μg/ml)	0.01	1.38
Cyanocobalemin (0.9 μg/ml)	0.01	1.55

To detect cobalamin (vitamin B_{12}) in the JCM 1112^T cell extract, a bioassay was performed as reported previously[S2]. *L. delbrueckii* ATCC 7830 was inoculated in vitamin B12-free assay media (Merck Co. Ltd.) supplemented with cell extracts of JCM 1112^T, *L. plantarum* WCFS1, saline solution, and cyanocobalamin solution. Each medium was incubated at 37°C for 10 hr. The culture growth was measured at the optical density at 560 nm. Quantification analyses using a cyanocobalamin standard curve indicated that JCM 1112^T produced approximately 0.7 μg of cobalamin/ml. No cobalamin was found in the JCM 1112^T cultural supernatant.

Supplementary Figure S1. Circular genome maps of *L. reuteri* JCM 1112^{T} and *L. fermentum* IFO 3956.

The outer scale is shown in megabases. Circle 1 (from the outside in), plus strand ORFs (red); Circle 2, minus strand ORFs (blue); Circle 3, transposases (orange), group II introns (light blue); Circle 4, tRNA genes (green), rRNA genes (brown); Circle 5, GC bias [(G-C)/(G+C) khaki indicates values > 0; purple indicates values < 0]; Circle 6, GC content [outward: higher values than the average]. The position of the *pdu-cbi-cob-hem* cluster is indicated by a shaded sector in *L. reuteri* JCM 1112^{T} (*pdu* genes, yellow; *cbi* genes, pink; *cob* genes, orange; *hem* genes, blue).

Supplementary Figure S2. Phylogenetic relationship between the *pdu* clusters from different sources.

The trees were based on concatenated amino acid sequence alignments of PduCDE homologues using ClustalW with a bootstrap trial of 1,000 and bootstrap values (%) are indicated at the nodes. An unrooted tree was generated using NJplot and scale bars represent the length of the branches. Genes are shown with arrows indicating the direction of transcription using the following colors; *pdu* including *gupCDE*: yellow; *cob*: orange; *pocR*: red; *eut*: green; other genes: white.

Supplementary Figure S3. Construction of pUC19-ΔgupCDE-ermAM for the generation of the *gupCDE* knockout mutant, LRΔ*gupCDE*.

A *gupCDE* knockout mutant (LRΔ*gupCDE*) of *L. reuteri* JCM 1112^{T} was constructed as follows. The erythromycin and ampicillin resistant genes from pIL253[S3] and pUC19 were used. Fragments for splice overlap extension PCR[S4] were amplified using the following primers;

ermAM-forward:

5'-GAATTACAATTTACAGCGGAAAACGAAATGATACACCAATCA-3'

ermAM-reverse:

5'-CATTGATTCGAACCTCTTTAGCTCCTTGGAAG-3'

gupC-*Sal*I-forward:
5'-ATATGTCGACCCAACCTGCTTTATTACTAACA-3'
gupC-*Sal*I-reverse:
5'-CATTTCGTTTTCCGCTGTAAATTGTAATTCAAGGTG-3'
gupE-*Bam*HI-forward:
5'-GCTAAAGAGGTTCGAATCAATGAAGCCACGC-3'
gupE-*Bam*HI-reverse:
3'-ATATGGATCCATTACATTCTACATAGCCCA-5'.
L. reuteri JCM 1112^T was transformed with pUC19-ΔgupCDE-ermAM via a Gene pulser (Bio-Rad Laboratories, Inc.) at 2.5 kV, 25 μF, and 200 Ω. Spontaneous resolution of a single crossover insertion into a double crossover resulted in LRΔ*gupCDE*.

Supplementary Figure S4. Time-dependent changes of 2D ^1H, ^{13}C-HSQC spectra of cultures metabolizing $^{13}C_3$-glycerol.
The wild-type *L. reuteri* JCM 1112^T (**A**) and mutant LRΔ*gupCDE* (**B**) were cultured in MRS broth supplemented with 200 mM $^{13}C_3$-glycerol and glucose. Chemical shifts of ^{13}C-labeled metabolites produced from $^{13}C_3$-glycerol are assigned by a combination of 2D-^{13}C-HSQC, 2D-^{13}C-edited HSQC, and 2D-^{13}C-HSQC TOCSY spectra. As shown in Supplementary Figure S5, δ^1H (ppm) and $\delta^{13}C$ (ppm) of 3-HPA-hydrate 1 (Hh-1), 3-HPA-hydrate 2 (Hh-2), and 3-HPA-hydrate 3 (Hh-3) were observed at 5.17 and 91.5, 2.43 and 42.6, and 3.79 and 61.6 at pH 7.0, respectively. After 7.5 hour-incubation, pH of MRS broth decline from 6.3 to 5.0 in this study. As shown in (A) at 7.5 hours, δ^1H (ppm) and $\delta^{13}C$ (ppm) of Hh-2 and Hh-3 were observed at 2.55 and 40.5, and 3.83 and 60.6 at pH 5.0, respectively. Although the spot derived from Hh-1 in ^1H chemical shift was detected in this study, the spot was not shown in (A) at 7.5 hours, because the range of ^1H chemical shifts shown from 4.0 to 1.5 ppm in this figure.

Supplementary Figure S5. Identification of 3-HPA and 3-HPA-hydrate.
(**A**) HSQC NMR spectrum. (**B**) ^{13}C-edited HSQC NMR spectrum. (**C**) ^{13}C-HSQC-TOCSY

NMR spectrum. A 100 mM-potassium phosphate buffer (pH = 7.0) was used for extraction of $^{13}C_3$-glycerol-derived metabolites both *in vitro* (Supplementary Fig. S4) and *in vivo* (Fig. 4). ^{1}H and ^{13}C-chemical shifts are referenced from the internal standard of DSS at 0 ppm. Each assignment was abbreviated: glycerol 1 (positive), G-1; glycerol 2 (negative), G-2; 3-HPA 1 (positive), H-1; 3-HPA (negative), H-2; 3-HPA (negative), H-3; 3-HPA-hydrate 1 (positive), Hh-1; 3-HPA-hydrate 2 (negative), Hh-2; 3-HPA-hydrate 3 (negative), Hh-3; 1,3-PDO 1 (negative), P-1; and 1,3-PDO 2 (negative), P-2, respectively. Contour levels of 3-HPA showed reduced signals. Purple, red, and black lines indicate H, Hh, and P correlations, respectively. Spots surrounded by red circles represent signals derived from 3-HPA-hydrate detected in (A) and (B). The spectra surrounded by squares represent the enlarged view of the 2D-NMR spectra in (A), (B), and (C).

Supplementary References

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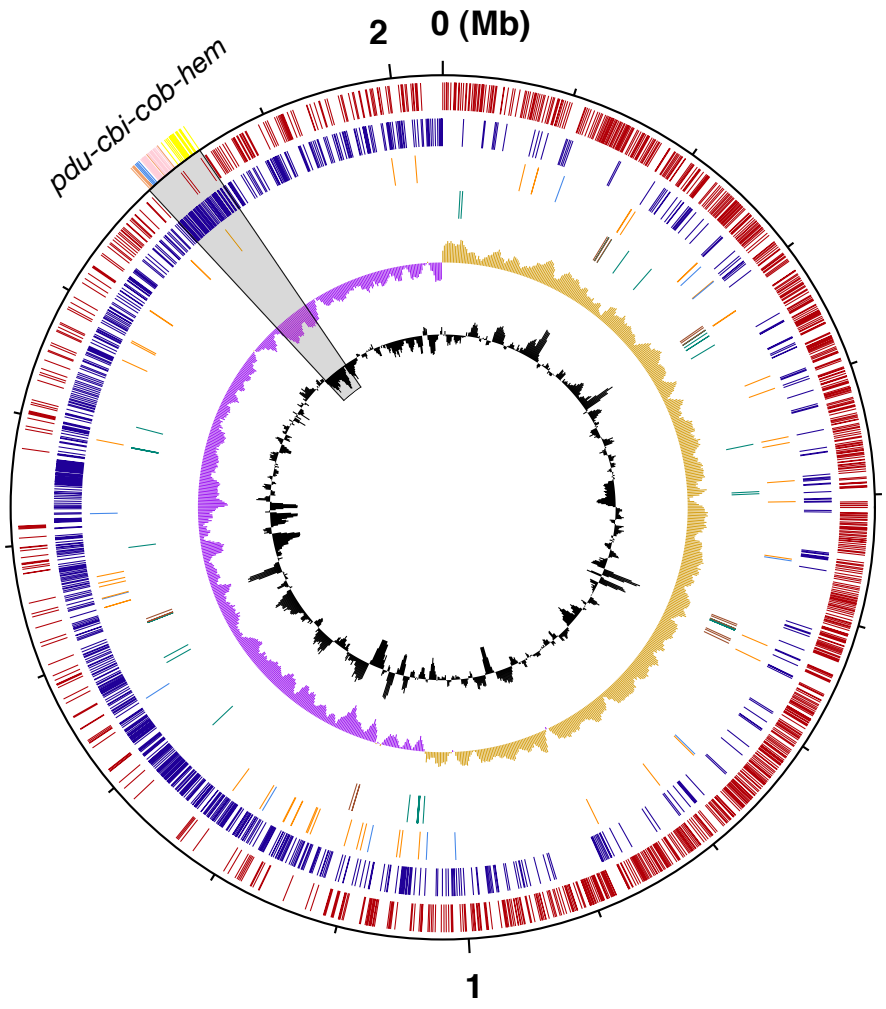
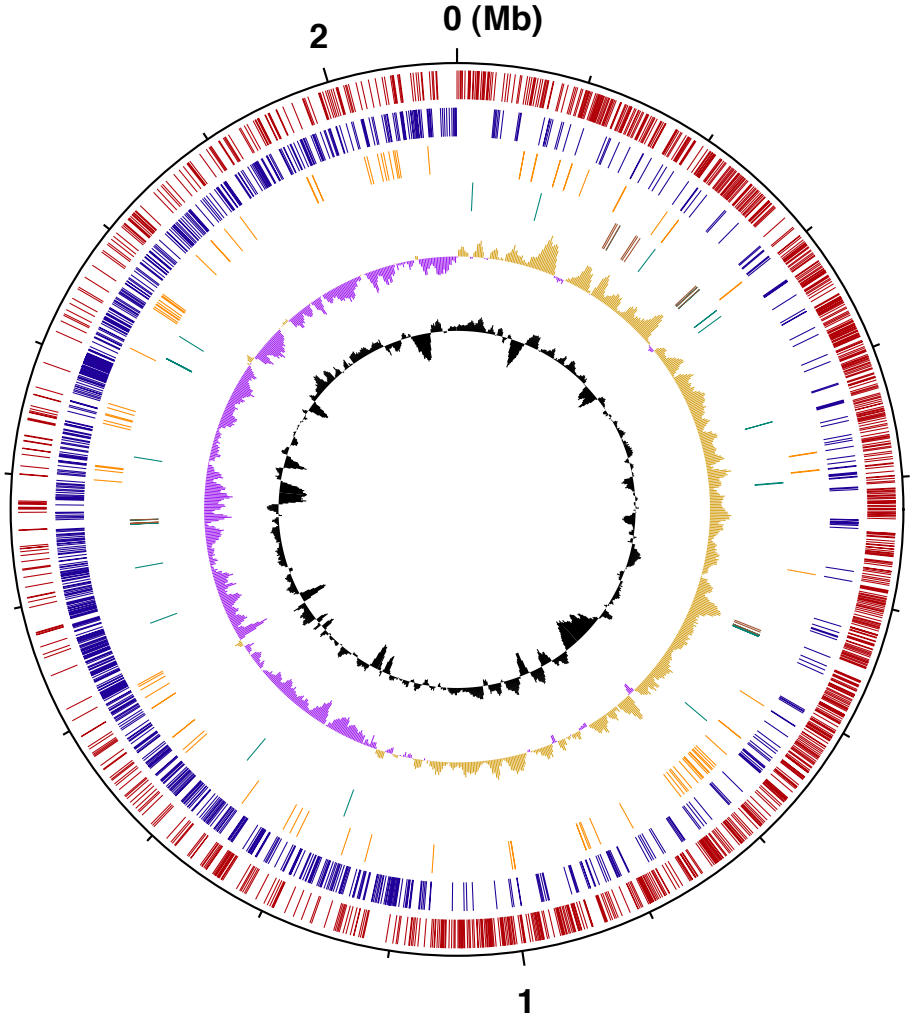
L. reuteri JCM 1112^{T}

2,039,414 bp

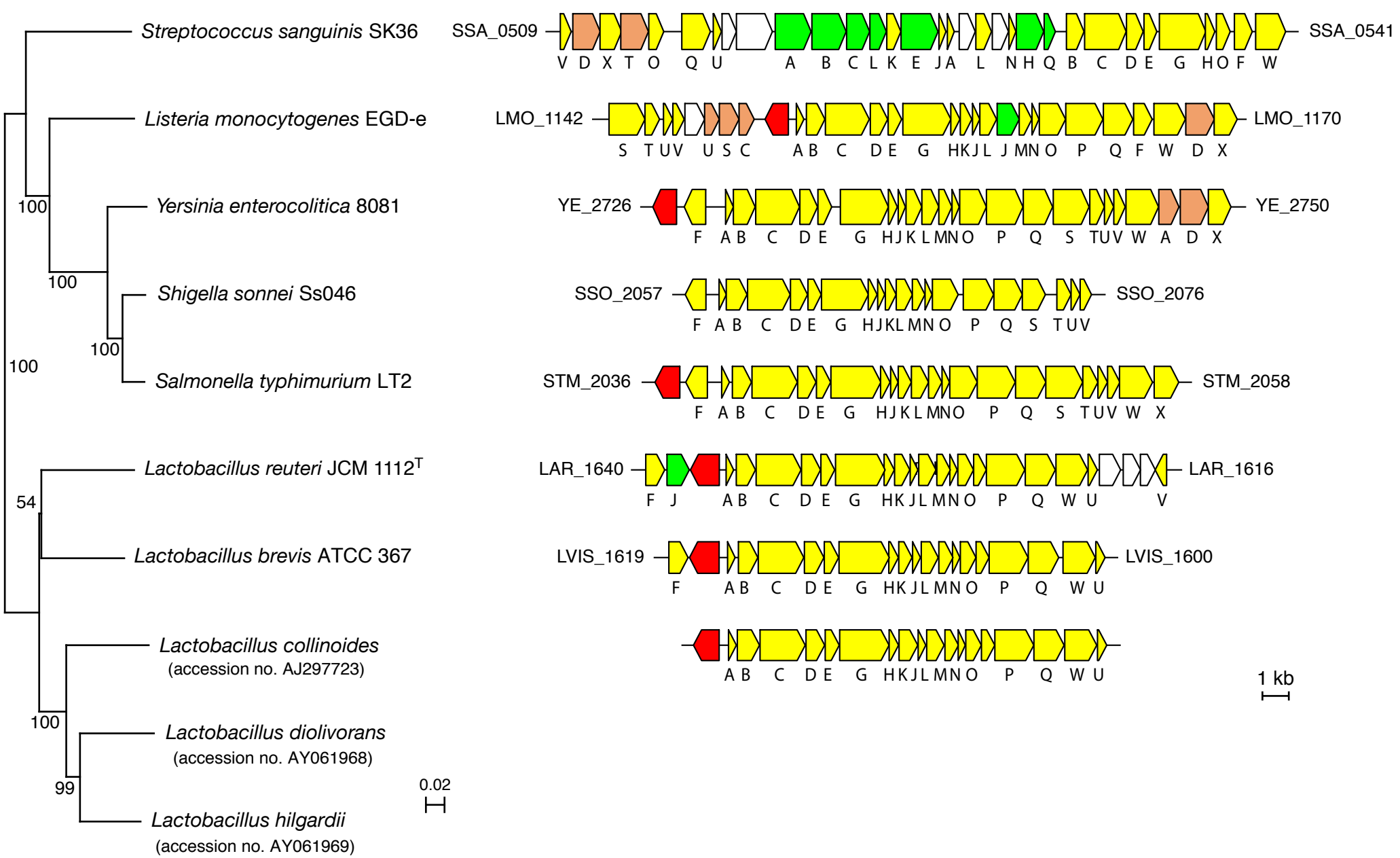


L. fermentum IFO 3956

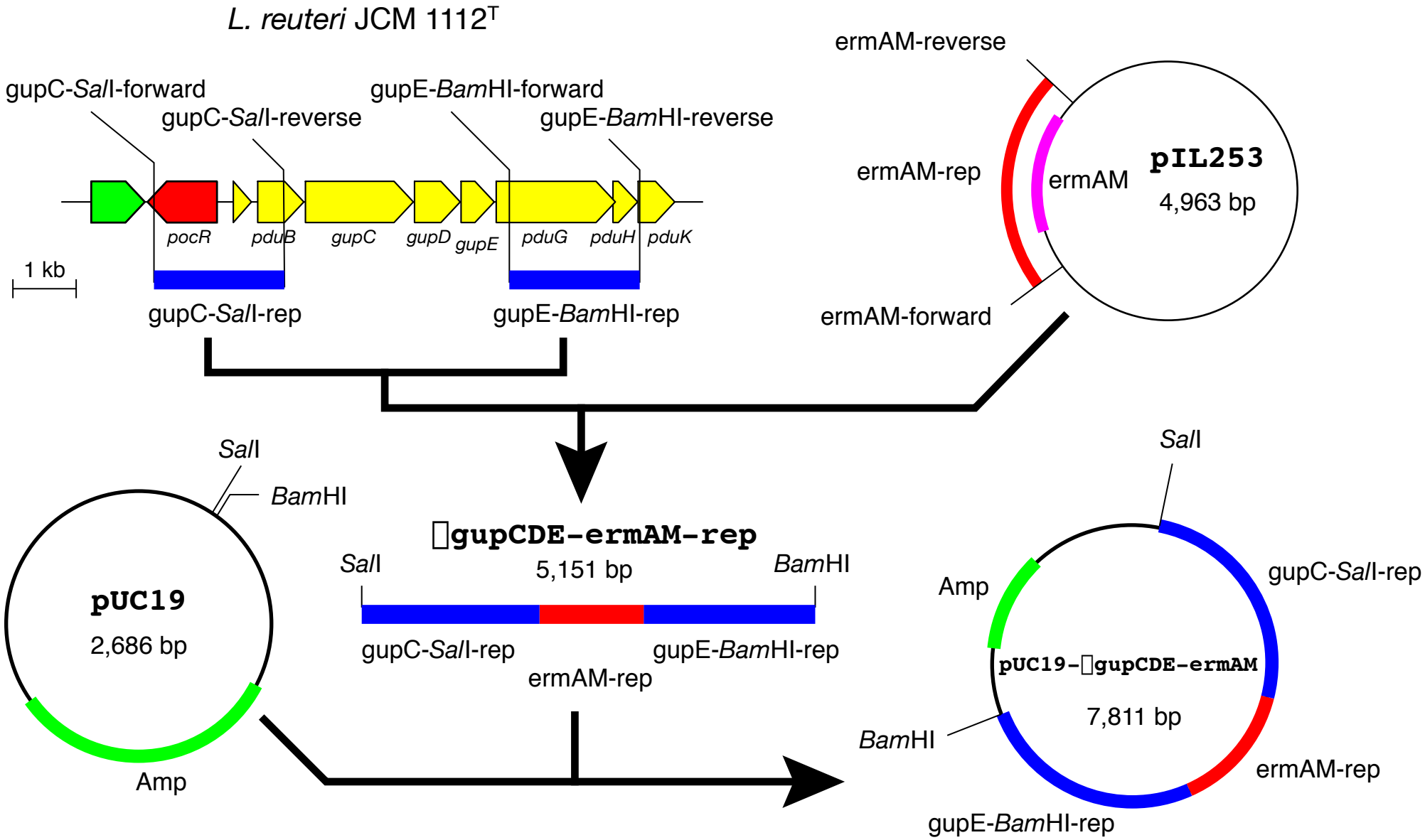
2,098,685 bp



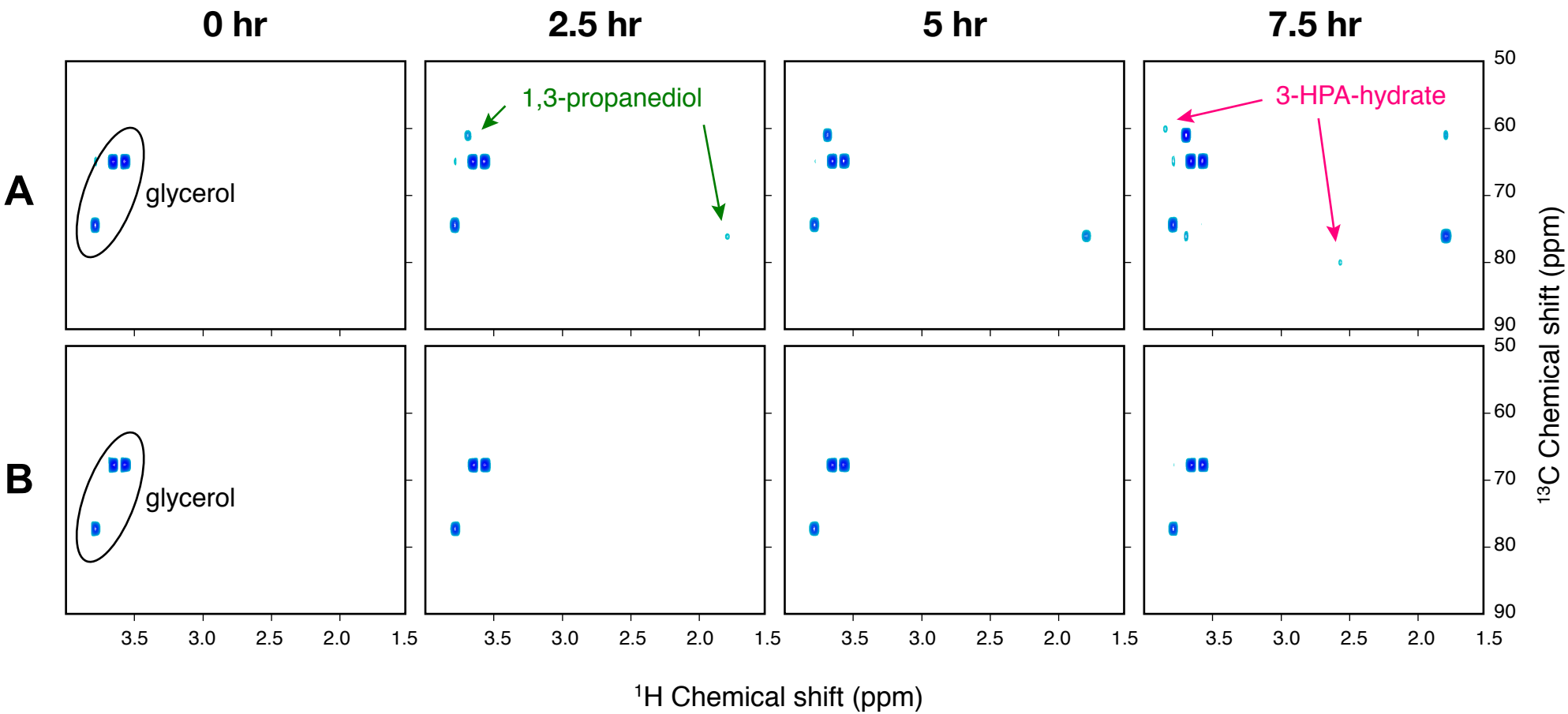
Supplementary Figure S1



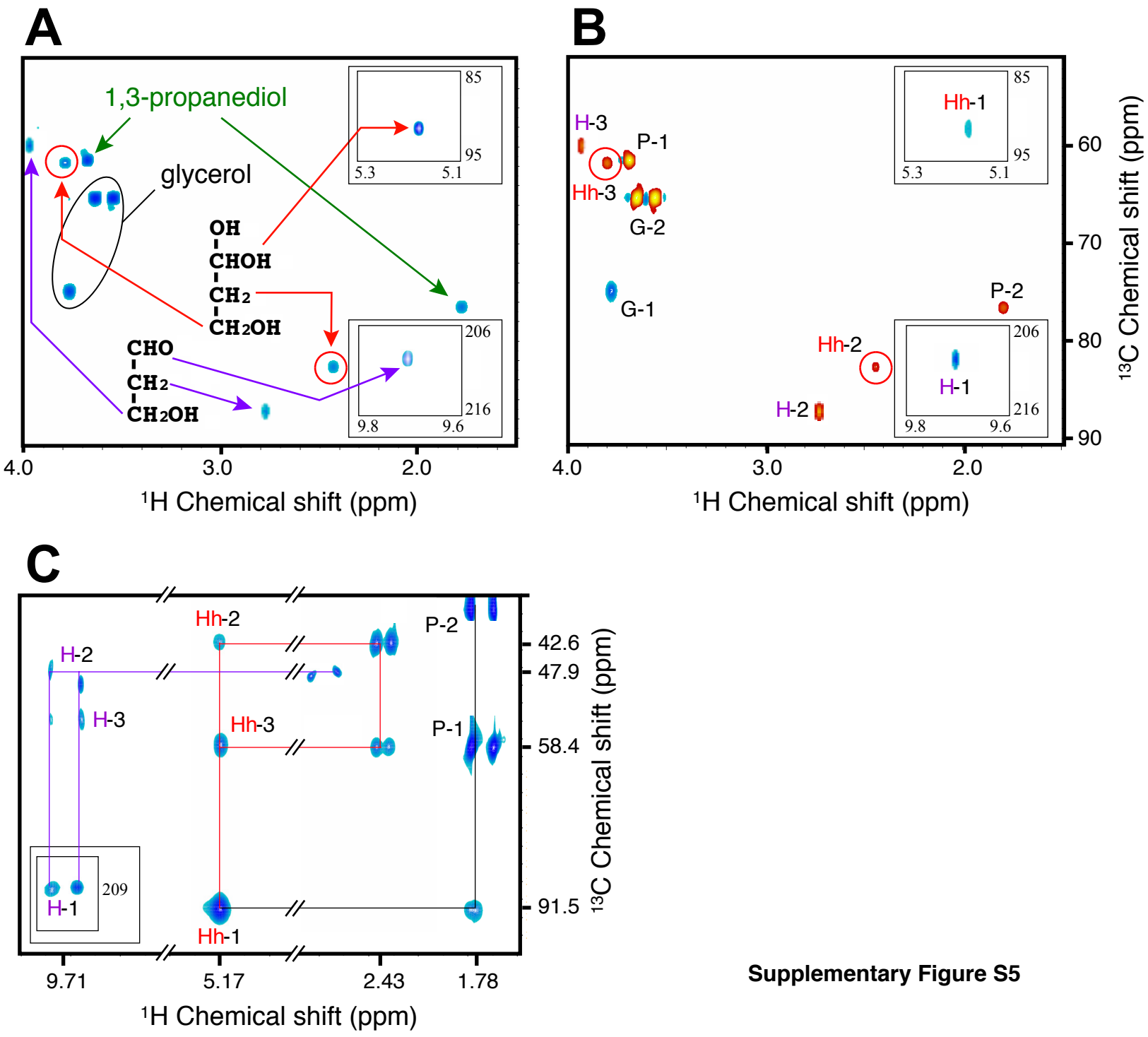
Supplementary Figure S2



Supplementary Figure S3



Supplementary Figure S4



Supplementary Figure S5