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Introduction

Streptococcus ruminantium, previously classified as *Streptococcus suis* serotype 33, is frequently isolated from ruminants, particularly cattle. Due to historical misclassification, *S. ruminantium* isolates may have been inaccurately identified as *S. suis*, which complicates our understanding of their epidemiology and pathogenic potential.

In this study, we identified ten *S. ruminantium* isolates from diseased or dead cattle. Notably, while some isolates corresponded to the classical serotype 33, seven others harbored distinct capsular polysaccharide (*cps*) loci.

Methods

Bacterial isolates were collected from diseased or dead cattle, each isolate from a different farm. Seven isolates were from calves, and all but one isolate from a rectal swab were cultured from lung or bronchoalveolar lavage. The other two isolates were also cultured from lungs, but from one bull and one heifer. The last isolate was from mastitic milk. The isolates were initially identified by MALDI-TOF as *S. suis* isolates. Whole genome sequences revealed their close relationship to *Streptococcus suis* serotype 33, which is now classified as *Streptococcus ruminantium*. A Neighbor-Joining phylogenetic tree was constructed from the core genome alignment of *S. suis* reference serotypes and our analyzed *S. ruminantium* isolates, and visualized using Jalview (Figure 1).

Clusters of *cps* genes were extracted and analyzed to investigate potential serotype diversity

Results and discussion

The average nucleotide identity (ANI) among the analyzed isolates ranged from 98.35% to 98.80%. Analysis of the *cps* gene clusters revealed potentially novel capsular type among *S. ruminantium* strains. The *cps* cluster of five isolates was similar to the *cps* cluster of serotype 33. For the other isolates, the *cps* cluster was similar to each other but significantly different from the *cps* cluster of serotype 33, suggesting a potential new serotype of *S. ruminantium* (Table 3). This finding suggests a broader *cps* diversity than previously recognized, indicating the presence of additional serotypes. Detailed classification based on *cps* loci may thus contribute to a better understanding of the pathogenicity and zoonotic potential of *S. ruminantium*.

Figure 1: Core genome alignment phylogenetic tree – Neighbor-joining method. *S. ruminantium* in red; *S. suis* in black.

Table 1: Pangenome genes summary

Genes	Description	Number
Core genes	(99% <= strains <= 100%)	1458
Soft core genes	(95% <= strains < 99%)	0
Shell genes	(15% <= strains < 95%)	1887
Cloud genes	(0% <= strains < 15%)	3245
Total genes	(0% <= strains <= 100%)	6590

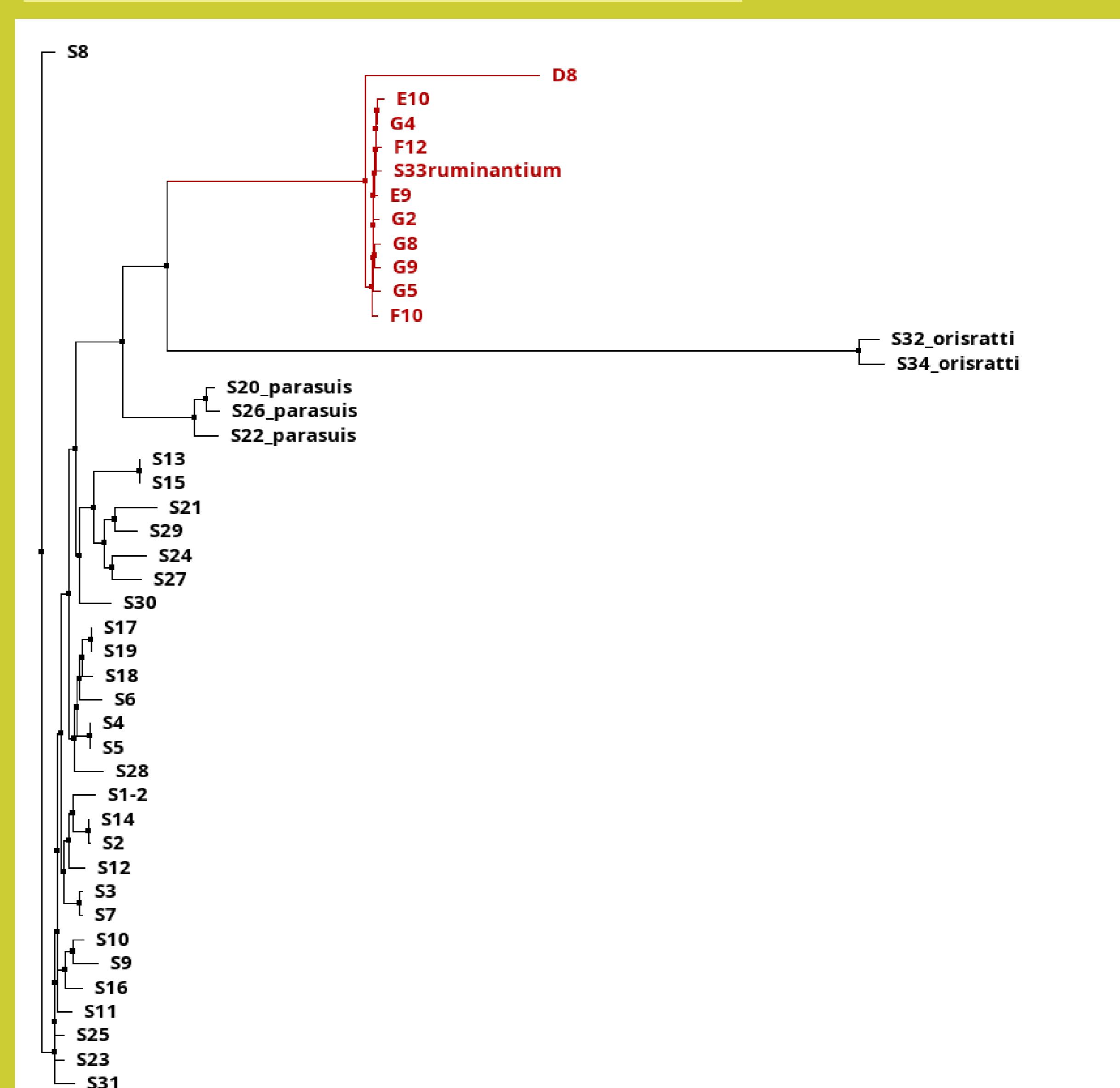


Table 2: Schematic representation of genes presence or absence between samples:

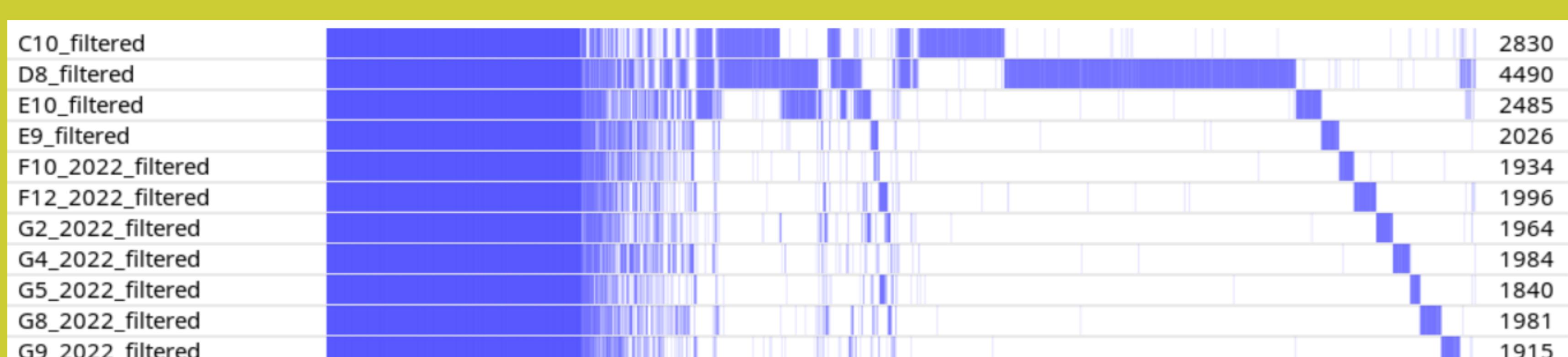


Table 3: Summary of genes in the *cps* locus. Genes were annotated using PROKKA.

S. ruminantium - reference strain (C10)			E10			G2, G9			G5			F12			
bp	gene	product	bp	gene	product	bp	gene	product	bp	gene	product	bp	gene	product	
1110	gff	UDP-galactopyranose mutase	1110	gff	UDP-galactopyranose mutase	1110	gff	UDP-galactopyranose mutase	1113	gff	UDP-galactopyranose mutase	1113	gff	UDP-galactopyranose mutase	
1392	hypothetical protein	1392	hypothetical protein	1392	hypothetical protein	1392	hypothetical protein	1461	murJ_1	lipid II flipase MurJ	1460	murJ_1	lipid II flipase MurJ		
1053	rfbB	dTDP-glucose 4,6-dehydratase	1053	rfbB	dTDP-glucose 4,6-dehydratase	1053	rfbB	dTDP-glucose 4,6-dehydratase	1158	hypothetical protein		1158	hypothetical protein		
720	ispD	2-C-methyl-D-erythritol 4-phosphate cytidylyltransferase	720	ispD	2-C-methyl-D-erythritol 4-phosphate cytidylyltransferase	720	ispD	2-C-methyl-D-erythritol 4-phosphate cytidylyltransferase	1062	rfbB	dTDP-glucose 4,6-dehydratase	1062	rfbB	dTDP-glucose 4,6-dehydratase	
975	hypothetical protein	975	hypothetical protein	975	hypothetical protein	975	hypothetical protein	720	ispD	2-C-methyl-D-erythritol 4-phosphate cytidylyltransferase	720	ispD	2-C-methyl-D-erythritol 4-phosphate cytidylyltransferase		
1311	hypothetical protein	228	hypothetical protein	216	tarI	Ribitol-5-phosphate cytidylyltransferase	216	tarI	Ribitol-5-phosphate cytidylyltransferase	1074	hypothetical protein		1074	hypothetical protein	
921	hypothetical protein	1293	hypothetical protein	1293	hypothetical protein	1293	hypothetical protein	918	hypothetical protein		918	hypothetical protein			
948	hypothetical protein	948	hypothetical protein	924	hypothetical protein	924	hypothetical protein	954	hypothetical protein		954	hypothetical protein			
816	wbbD	UDP-Gal:alpha-D-GlcNAc-diphosphoheptadecaprenol beta-1,3-galactosyltransferase	816	wbbD	UDP-Gal:alpha-D-GlcNAc-diphosphoheptadecaprenol beta-1,3-galactosyltransferase	816	wbbD	UDP-Gal:alpha-D-GlcNAc-diphosphoheptadecaprenol beta-1,3-galactosyltransferase	816	wbbD	UDP-Gal:alpha-D-GlcNAc-diphosphoheptadecaprenol beta-1,3-galactosyltransferase	816	wbbD	UDP-Gal:alpha-D-GlcNAc-diphosphoheptadecaprenol beta-1,3-galactosyltransferase	
603	pglC	Undecaprenyl phosphate N,N'-diacytibacillosamine 1-phosphate transferase	603	pglC	Undecaprenyl phosphate N,N'-diacytibacillosamine 1-phosphate transferase	603	pglC	Undecaprenyl phosphate N,N'-diacytibacillosamine 1-phosphate transferase	603	pglC	Undecaprenyl phosphate N,N'-diacytibacillosamine 1-phosphate transferase	603	pglC	Undecaprenyl phosphate N,N'-diacytibacillosamine 1-phosphate transferase	
717	hypothetical protein	717	hypothetical protein	717	hypothetical protein	717	hypothetical protein	717	hypothetical protein		717	hypothetical protein			
1827	pglF	UDP-N-acetyl-alpha-D-glucosamine C6 dehydratase	1827	pglF	UDP-N-acetyl-alpha-D-glucosamine C6 dehydratase	1827	pglF	UDP-N-acetyl-alpha-D-glucosamine C6 dehydratase	1827	pglF	UDP-N-acetyl-alpha-D-glucosamine C6 dehydratase	1827	pglF	UDP-N-acetyl-alpha-D-glucosamine C6 dehydratase	
732	cpsB	Tyrosine-protein phosphatase CpsB	732	cpsB	Tyrosine-protein phosphatase CpsB	732	cpsB	Tyrosine-protein phosphatase CpsB	732	cpsB	Tyrosine-protein phosphatase CpsB	732	cpsB	Tyrosine-protein phosphatase CpsB	
696	cpsD	Tyrosine-protein kinase CpsD	696	cpsD	Tyrosine-protein kinase CpsD	696	cpsD	Tyrosine-protein kinase CpsD	696	cpsD	Tyrosine-protein kinase CpsD	696	cpsD	Tyrosine-protein kinase CpsD	
690	ywqC	putative capsular polysaccharide biosynthesis protein YwqC	690	ywqC	putative capsular polysaccharide biosynthesis protein YwqC	690	ywqC	putative capsular polysaccharide biosynthesis protein YwqC	690	ywqC	putative capsular polysaccharide biosynthesis protein YwqC	690	ywqC	putative capsular polysaccharide biosynthesis protein YwqC	
1440	tagU_1	Polyisoprenyl-teichoic acid-peptidoglycan teichoic acid transferase TagU	1440	tagU_1	Polyisoprenyl-teichoic acid-peptidoglycan teichoic acid transferase TagU	1440	tagU_1	Polyisoprenyl-teichoic acid-peptidoglycan teichoic acid transferase TagU	1440	tagU_2	Polyisoprenyl-teichoic acid-peptidoglycan teichoic acid transferase TagU	1440	tagU_2	Polyisoprenyl-teichoic acid-peptidoglycan teichoic acid transferase TagU	
744	yaaA	Peroxide stress resistance protein YaaA	744	yaaA	Peroxide stress resistance protein YaaA	744	yaaA	Peroxide stress resistance protein YaaA	744	yaaA	Peroxide stress resistance protein YaaA	744	yaaA	Peroxide stress resistance protein YaaA	

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1110	gff	UDP-galactopyranose mutase	1107	gff	UDP-galactopyranose mutase	1107	gff	UDP-galactopyranose mutase	1113	gff	UDP-galactopyranose mutase	1113	gff	UDP-galactopyranose mutase	
1392	hypothetical protein	1392	hypothetical protein	1392	hypothetical protein	1392	hypothetical protein	1461	murJ_1	lipid II flipase MurJ	1460	murJ_1	lipid II flipase MurJ		
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975	hypothetical protein	975	hypothetical protein	975	hypothetical protein	975	hypothetical protein	720	ispD	2-C-methyl-D-erythritol 4-phosphate cytidylyltransferase	720	ispD	2-C-methyl-D-erythritol 4-phosphate cytidylyltransferase		
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816	wbbD	UDP-Gal:alpha-D-G													