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Research article

Structure of Genetic Loci for Capsular Polysaccharide Biosynthesis in *Streptococcus parauberis* isolated from Japanese flounder

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ABSTRACT—*Streptococcus parauberis* is a pathogen of streptococcosis in turbot *Scophthalmus maximus* and the Japanese flounder *Paralichthys olivaceus*. *S. parauberis* isolates from diseased Japanese flounder have been classified into several serological phenotypes. In this study we analyzed the DNA sequence of the genetic locus for capsular polysaccharide (CPS) biosynthesis of *S. parauberis* KRS02083 (subserotype Ia), NUF1003 (subserotype Ib), NUF1071 (subserotype Ic), NUF1032 (serotype II), 2007-1 (nontypeable/PFGE cluster I) and NUF1095 (nontypeable/PFGE cluster III) to elucidate the genetic basis for serological diversity. As a result, three kinds of *cps* locus were revealed among serotypes and subserotypes, namely the loci for subserotype Ia, subserotypes Ib/Ic and serotype II. The genetic structure of *cps* loci suggests that the capsules of *S. parauberis* are synthesized through the Wzy-dependent pathway. Subserotypes Ib and Ic possessed the same genetic structure, although single-base substitution at several regions or insertion of an *IS* (insertion sequence) element was found in subserotype Ic. The nontypeable strains, which agglutinated with both serotypes I and II antisera, possessed the same genetic structure as subserotype Ib/Ic or serotype II with single-base substitution at several regions.

Key words: *Streptococcus parauberis*, streptococcosis, serotype, capsular polysaccharide biosynthesis, *cps* locus, structure, *Paralichthys olivaceus*

Polysaccharide capsules are particular structure found on the cell surface of many bacterial species. They are usually regarded as crucial virulence factors and have antigenicity, which can be used for serotyping of bacteria. The diversity of capsular polysaccharide (CPS) structure due to a variety of sugars and glycosidic linkages is reflected in the differences of serotype (Okura *et al*., 2013). Generally, the CPS biosynthetic genes are clustered at a single locus (*cps* locus) on the chromosome (Roberts, 1996) with a rare exception (Patrick *et al*., 2010). The biosynthesis of CPS requires a complex pathway, where there are two major pathways termed synthase-dependent pathway and Wzy-dependent pathway, which are responsible for polymerization of individual sugars in a processive reaction and discrete repeat units in a nonprocessive reaction, respectively, in Gram-positive bacteria (Yother, 2011).

Streptococcus parauberis, a Gram-positive coccus, can cause mastitis in cows (Williams and Collins, 1990) and is known as a pathogen in turbot *Scophthalmus maximus* and the Japanese flounder *Paralichthys olivaceus* (Domeénech *et al*., 1996; Kanai *et al*., 2009). Our previous studies demonstrated that the isolates from diseased flounder were classified into two serotypes, designated I and II, and that the serotype I was subdivided into three subserotypes, designated Ia, Ib and Ic, while nontypeable strains, which agglutinated with both serotypes I and II antisera, were also found (Kanai *et al*., 2009, 2015). It is suggested that the serological diversity arise from difference of CPS and that the nontypeable strains do not produce CPS or produce a negligible amount of CPS on their cell surface (Kanai *et al*., 2009). The existence of serological diversity suggests the presence of distinct features of *cps* locus according to serotypes of this pathogen. To investigate the correlation between the serotypes and structure of *cps* loci of *S. parauberis*, we sequenced and analyzed the *cps* loci of the representative strain of each serotype and two nontypeable strains. The possible biosynthetic pathway and mechanisms by which the diversity arises in *S. parauberis* were also discussed.

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Materials and Methods

Bacterial strains and genomic DNA isolation

For DNA sequencing, four *S. parauberis* strains, KRS02083, NUF1003, NUF1071 and NUF1032, were selected as the representatives of subserotypes Ia, Ib and Ic and serotype II, respectively. Two nontypeable (NT) strains, 2007-1 and NUF1095, which belonged to the clusters I and III in *sma*I-PFGE analysis (Kanai *et al*., 2015), respectively, were also included (Table 1). For PCR scanning (verification of the structure of the *cps* loci by amplifying the parts of the entire *cps* locus), remaining 182 *S. parauberis* strains were used (Kanai *et al*., 2015). All the strains were cultured on Todd Hewitt (Difco) agar at 27°C for 24 h, and genomic DNA was prepared using Wizard Genomic DNA Purification kit (Promega) according to the manufacturer's instructions.

Sequencing of the cps *loci of* S. parauberis *NUF1003 and NUF1032*

Initial PCR amplification and sequencing: The primer pair SP1F (5´-TATYAGYGGTATTGATAC) and SP2R (5´-GGTGGRGTATCRATGAT) was designed from the conserved region of *cps* loci of *S. agalactiae* serotype Ia (GenBank accession no., AB028896) and III (AF163833), *S. thermophilus* (DQ393658) and *S. iniae* (AY904444), and the initial PCR was conducted to amplify a partial sequence of the *cps* loci of *S. parauberis* NUF1003 and NUF1032. The PCR was performed on C1000 Thermal Cycler (Bio-Rad) using *TaKaRa Ex Taq* Hot Start Version (Takara). The PCR reaction mixture (a total volume of 50 μL) contained 5 μL of ×10 Ex Taq buffer (Mg^{2+} plus), 4 μL of dNTP mixture (0.2 mM each), SP1F and SP2R primers (0.5 μM each), 1 μL of template DNA and 1.25 U of *Ex Taq* DNA polymerase. The condition of PCR amplification was denaturation at 95°C for 5 min, 30 cycles of denaturation at 95°C for 30 s, annealing at 55°C for 30 s and extension at 72°C for 3 min. The PCR products were cloned into pGEM-T Easy vector using pGEM-T Easy Vector System (Promega) with *Escherichia coli* JM109 competent cells (Promega), and three clones per strain were sequenced using BigDye Terminator v3.1 Cycle Sequencing kit and ABI PRISM 3130xl Genetic Analyzer (Applied Biosystems).

Inverse PCR and sequencing: The adjacent upstream and downstream regions of the sequenced site of the *cps* locus were amplified and sequenced successively using inverse PCR technique (Ochman *et al*., 1988). Briefly, genomic DNA was digested with each of restriction enzymes (*Bam*HI, *Bgl*II, *Eco*RI, *Eco*RV, *Hin*dIII, *Kpn*I, *Pst*I, *Pvu*II, *Sph*I) and self-ligated using DNA Ligation kit ver. 2.1 (Takara). Inverse PCR was performed using *TaKaRa Ex Taq* Hot Start Version with the self-ligated (looped) DNA and oppositely directed primer pair designed from the sequenced region of the *cps* locus by the PCR condition mentioned above, except that the extension time was changed to 5 min. The PCR products obtained were cloned and sequenced as above. Inverse PCR was repeated using primers newly designated from the sequenced region until the entire sequence data of *cps* locus was obtained. DNASIS program (Hitachi Software Engineering) was used for the DNA sequence assembling.

Sequencing of the cps *loci of* S. parauberis *KRS02083*, *NUF1071*, *2007-1 and NUF1095*

Primer pairs were designed from the sequence data of NUF1003 and NUF1032, and PCRs were performed to amplify the parts of entire *cps* loci of KRS02083, NUF1071, 2007-1 and NUF1095 using PrimeSTAR HS DNA Polymerase (Takara). The PCR products were sequenced directly using BigDye Terminator v3.1 Cycle Sequencing kit and ABI PRISM 3130xl Genetic Analyzer (Applied Biosystems). In the case of KRS02083, inverse PCR technique was also applied, since several parts could not be amplified.

Analysis of sequence data

Open reading frames (ORFs) were predicted using NCBI ORF finder (http://www.ncbi.nlm.nih.gov/gorf/orfig.html) which could link to the BLAST network server (http://blast.ncbi.nlm.nih.gov/Blast.cgi). All predicted genes were named in alphabetical order in each sero-

Table 1. Representative *Streptococcus parauberis* strains used for *cps* locus analyses

Strain	Origin		Serotype*	Affiliation in *Sma*I-PFGE analysis*	*cps* locus		
	Prefecture	Year			Length (bp)	No. of ORF	G+C content (%)
KRS02083	Kagawa	2002	Ia	Cluster II	18,910	19	31.14
NUF1003	Shimane	2004	Ib	Cluster I	19,334	18	31.07
NUF1071	Nagasaki	2008	Ic	Cluster I	19,334	18	31.07
NUF1032	Nagasaki	2006	II	Cluster III	19,920	19	31.51
2007-1	Oita	2007	NT	Cluster I	19,334	18	31.07
NUF1095	Nagasaki	2009	NT	Cluster III	19,920	19	31.51

* Kanai *et al*., 2015; NT, nontypeable; All the strains were isolated from diseased Japanese flounder.

type (e.g. *cpsA-cpsR*). ClustalW2.1 network version on DDBJ (http://clustalw.ddbj.nig.ac.jp/index.php?lang=en) was used for nucleotide and amino acid sequence alignments.

PCR scanning

To verify whether the structure of *cps* loci of the remaining 182 *S. parauberis* strains was the same as the representative strains of respective serotype, PCR that spanned the entire region of *cps* locus was conducted with primer pairs listed in Table 2. The primers

Table 2. Primers used for PCR scanning of *cps* locus

Primer	Sequence (5´–3´)	Region	Approximate size of amplicon (bp)	Annealing temperature (°C)
Sp-cps21	GACATCACGGTTATAGTC	*LysR-cpsB*	2,600	55
Sp-cpsR	GTTCGATATGCGCGAC			
5sp-IP6	CTAGATGATCAGAGTGTC	*cpsA-cpsD*	1,600	51
3sp-IP1	GATGTTAGTCCGAATGGAG			
I-1-3sp2	GACCGCGTGAAACGCCCAG	*cpsC-cpsE*	2,300	61
Sp-cps26	TGAATGACAAGACGACTAGC			
3sp-IP5	GTACTCGATTAAGCGAC	*cpsE-cps1aG*	3,000	49
Sp-cps47	CTGATAATTCCCGATCAATG			
Sp-cps32	ACTAGCAGCTGAGAATGG	*cps1aG-cps1aI*	1,900	55
Sp-cps48	GCCATCAGCAATAGAAATAC			
Sp-cps38	ATCCTTCTGTAGTATTAGGC	*cps1aH-cps1aK*	3,300	51
Sp-cps49	GAACCTTGTATAACGTCAAC			
Sp-cps41	CTAACGAAATCGGAGCTG	*cps1aK-cps1aM*	2,200	55
Sp-cps50	TGAGACTACTGAACCTGCAG			
Sp-cps45	TACGTGGGATTCTAATC	*cps1aL-cps1aN*	2.900	51
Sp-cps42	GATTTTACGAATTCTTGTTC			
Sp-cps51	ATTGCAACAGCAGCTATTTC	*cps1aN-cps1aP*	2,300	55
Sp-cps52	ACACCAGCAGAAATTAGTTC			
Sp-cps36	TAGCAGCACAAGCAGGAG	*cps1aP-cps1aR*	2,600	55
3sp-IP85	CACAATGATTTTCAAAGATG			
3sp-IP7	CATGGTCTCAACTGAC	*cpsE-cps1bG*	2,300	50
3sp-IP38	AGGTCTAACTTCATGTCTAC			
3sp-IP17	GAAAGAGGACTTTGCTTGCC	*cps1bF-cps1bI*	2,200	59
3sp-IP36	GTCAAAGTGTCATCGACAATCTC			
3sp-IP23	TACGGTGCAATAGTTGCC	*cps1bI-cps1bK*	2,800	56
3sp-IP45	GTTTTTACCTAATCTATCCTTACTA			
3sp-IP39	GAACATCATCATATTGATCG	*cps1bK-cps1bM*	2,700	54
3sp-IP53	TACATACCACAAACATAATCC			
3sp-IP50	AGTGCTCTCGTAATAGCTCTGG	*cps1bM-cps1bO*	2,800	56
Sp-cps52	ACACCAGCAGAAATTAGTTC			
3sp-IP58	GAATGACTATTACGATGTCTC	*cps1bO-cps1bQ*	2,700	54
3sp-IP85	CACAATGATTTTCAAAGATG			
3sp-IP5	GTACTCGATTAAGCGAC	*cpsE-cps2F*	2,200	53
Sp-cps7	GGACATACCATTTGCAGTA			
3sp-IP14	GAATCAGGAATCAGACCTGG	*cps2E-cps2G*	1,900	58
3sp-IP72	TGAACATTGCAGGCTATACC			
Sp-cps8	TAGCTGATGGTGCTCATG	*cps2G-cps2I*	1,500	55
3sp-IP67	GTACTTGCCGAAACTATTGTG			
3sp-IP61	GGTAGAGAAATGAATGAATATCG	*cps2H-cps2J*	2,100	55
3sp-IP68	TTCAGGTCCACCACCGATG			
3sp-IP65	GGAGATTGTAATGAGGTAAATC	*cps2J-cps2L*	2,200	55
3sp-IP66	CACCAATATACACAGTCTAAATC			
Sp-cps23	GCAAATGATTCAGATGA	*cps2L-cps2N*	2,300	55
Sp-cps53	CTGGTATGCCACCAACAATC			
3sp-IP78	TGAGCATCGAACAGATACAG	*cps2N-cps2P*	2,900	55
Sp-cps12	TTTTGAATCATTGAAGTCG			
3sp-IP86	CTTAATGATACATTTGCAACAG	*cps2P-cps2R*	2,000	55
3sp-IP85	CACAATGATTTTCAAAGATG			

were designed so that the neighbored PCR products overlapped each other. The PCR was performed on C1000 Thermal Cycler (Bio-Rad) using *TaKaRa Ex Taq* Hot Start Version (Takara) and the condition mentioned above, but the annealing temperature listed in Table 2 and extension time of 1 min/kb were employed.

Nucleotide sequence accession numbers

The nucleotide sequences of six *S. parauberis cps* loci obtained in this study were deposited in the DDBJ/EMBL/GenBank databases under accession numbers from LC060252 to LC060257 for KRS02083, NUF1003, NUF1071, NUF1032, 2007-1 and NUF1095, respectively.

Results

General features of the cps *loci of the representative strains*

By PCR amplification, cloning and sequencing, the constitution of *cps* loci of six representative strains became apparent (Fig. 1). The length, number of ORF and G+C content of the loci are shown in Table 1. The function of each gene was predicted based on the homology of its deduced amino acid sequence with those on the database (Tables 3 and 4). In all the six strains, predicted genes are orientated in the same direction except the first gene, and cassette-like structure was observed, i.e., conserved regions flanked serotype-specific genes. At the upper conserved region of the *cps* locus, there were five regulatory genes, *LysR* and *cpsABCD* (also known as *wzg*, *wzh*, *wzd* and *wze*), and one processing (epimerase/short-chain dehydrogenase) gene (*cpsE*) sequentially, which

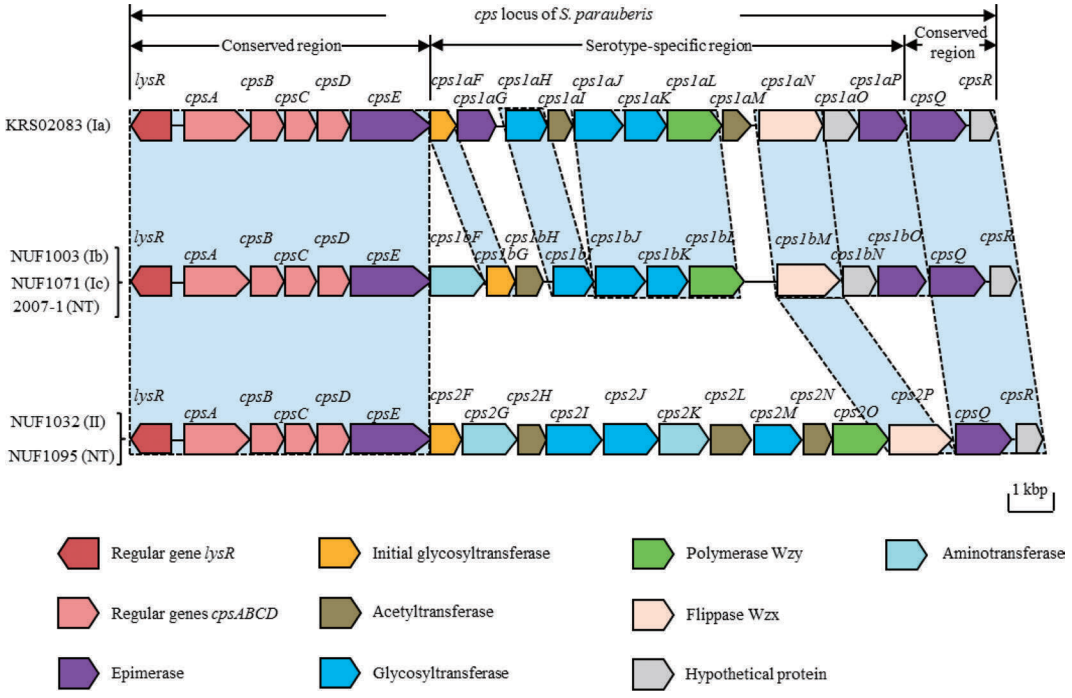

Fig. 1. Structure of *cps* loci of *S. parauberis* representative strains. Putative functions of genes are differentiated using different colors. Shaded regions indicate the genes whose deduced amino acid sequences showed over 30% identity among them.

Table 3. Proteins of other bacterial species homologous to deduced amino acid sequences of the genes in the conserved region

Gene	Category	Protein showing the highest identity			Identity
		Organism	Product	Accession no.	
lysR	Regulator	*Streptococcus uberis*	LysR family transcriptional regulator	WP_037627683	91%
cpsA		*Streptococcus uberis*	LytR family transcriptional regulator	WP_037627066	79%
cpsB		*Streptococcus uberis*	Tyrosine protein phosphatase	WP_037627064	92%
cpsC		*Streptococcus uberis*	Capsular biosynthesis protein CpsC	WP_037627063	87%
cpsD		*Streptococcus porcinus*	Tyrosine-protein kinase	WP_003085167	81%
cpsE	Epimerase	*Streptococcus pseudoporcinus*	Short-chain dehydrogenase	WP_007893078	80%
cpsQ		*Streptococcus uberis*	UDP-glucose 6-dehydrogenase	WP_046393482	86%
cpsR	Hypothetical	*Streptococcus uberis*	Hypothetical protein	WP_037627032	89%

Table 4. Proteins of other bacterial species homologous to deduced amino acid sequences of the genes in the serotype-specific region

Gene	Category	Protein showing the highest identity			Identify
		Organism	Product	Accession no.	
cps1aF	Initial glycosyltransferase	*Streptococcus uberis*	UDP-phosphate galactose phosphotransferase	WP_012658534	88%
cps1bG		*Lachnospiraceae* bacterium	UDP-galactose phosphate transferase	WP_009261689	67%
cps2F		*Streptococcus suis*	initial sugar transferase	FAA00872	60%
cps1aL	Polymerase	*Megasphaera sp.*	O-antigen ligase	WP_023052747	25%
cps1bL		*Clostridium sp.*	O-antigen polymerase	WP_022113405	25%
cps2O		*Streptococcus pseudoporcinus*	oligosaccharide repeat unit polymerase	WP_007893424	75%
cps1aN	Flippase	*Lactobacillus paraplantarum*	flippase Wzx	CDF77686	41%
cps1bM					42%
cps2P					36%
cps1aH	Glycosyltransferase	*Streptococcus uberis*	glycosyltransferase	WP_012658532	81%
cps1bI					61%
cps1aJ		*Acetobacterium woodii*	glycosyltransferase family 1	WP_041668735	48%
cps1bJ					46%
cps1aK		*Fibrobacter succinogenes*	glycosyltransferase family 2	WP_014546699	38%
cps1bK					39%
cps2I		*Streptococcus pseudoporcinus*	glycosyltransferase family 1	WP_007893181	77%
cps2J		*Streptococcus pseudoporcinus*	glycosyltransferase family 1	WP_007893376	70%
cps2M		*Bacteroides sp.*	glycosyltransferase, group 2 family	EFI06947	49%
cps1aI	Acetyltransferase	*Streptococcus uberis*	maltose O-acetyltransferase	WP_046388669	64%
cps1aM		*Clostridium akagii*	acetyltransferase	WP_035795827	49%
cps1bH		*Ruminococcus albus*	acetyltransferase	WP_024858893	56%
cps2N		*Streptococcus pseudoporcinus*	acetyl transferase	WP_007893375	65%
cps2H		*Streptococcus didelphis*	GNAT family acetyltransferase	WP_018366072	63%
cps2L		*Streptococcus pseudoporcinus*	GNAT family acetyltransferase	WP_007893003	80%
cps1aG	Epimerase	*Streptococcus uberis*	NAD-dependent epimerase	WP_046392145	72%
cps1aP		*Streptococcus porcinus*	NAD dependent epimerase/dehydratase family protein	WP_003085154	83%
cps1bO					84%
cps1bF	Aminotransferase	*Streptococcus suis*	aminotransferase DegT	WP_029175971	81%
cps2G		*Streptococcus suis*	aminotransferase	AKE79842	68%
cps2K		*Butyrivibrio proteoclasticus*	aminotransferase	WP_026662038	69%
cps1aO	Hypothetical	*Streptococcus equinus*	hypothetical protein	WP_033152408	50%
cps1bN					45%

were conserved with high sequence identity among all the six strains (> 99%). Each of two genes, *cpsQ* and *cpsR*, at the lower conserved region also showed high identity among the strains (> 96%). In the serotype-specific region, genes that encode the initial glycosyltransferase, polysaccharide polymerase (*wzy*) and flippase (*wzx*) were present at different locations along with genes encoding enzymes such as glycosyltransferase, acetyltransferase, aminotransferase and modifying enzymes (Fig. 1). As shown in Tables 3 and 4, most of the genes in the conserved region showed the highest identity with those of *Streptococcus uberis*, while the genes in the serotype-specific region did not necessarily show the high identity with them.

Comparison of the six cps *loci*

The nucleotide sequences of serotype-specific regions of NUF1003 (subserotype Ib), NUF1071 (subserotype Ic) and 2007-1 (NT type, *sma*I-PFGE Cluster I) were almost identical, and those of NUF1032 (serotype II) and NUF1095 (NT type, *sma*I-PFGE Cluster III) was also almost identical. The difference between NUF1003 and NUF1071 was only two single-base substitutions found in *cps1bM* and *cps1bN*. Similarly, the difference between NUF1003 and 2007-1 was three single-base substitutions in *cpsC*, *cps1bG* and *cps1bM*, and the difference between NUF1032 and NUF1095 was two single-base substitutions in *cpsC* and *cps2G* (data not shown). From these it is suggested that there are three kinds of *cps* locus among serotypes and subserotypes in *S. parauberis*, namely the loci for subserotype Ia, subserotypes Ib/Ic and serotype II (Fig. 1).

Sequence homology of the deduced amino acid

Table 5. Homology of deduced amino acid sequences between the genes in the serotype-specific region of the *cps* loci

Category	Predicted protein	Identity	Category	Predicted protein	Identity
Initial glycosyltransferase	Cps1aF – Cps1bG	40%	Polymerase Wzy	Cps1aL – Cps1bL	31%
	Cps1aF – Cps2F	18%		Cps1aL – Cps2O	10%
	Cps1bG – Cps2F	23%		Cps1bL – Cps2O	11%
Glycosyltransferase	Cps1aH – Cps1bI	60%	Flippase Wzx	Cps1aN – Cps1bM	72%
	Cps1aH – Cps2I	9%		Cps1aN – Cps2P	37%
	Cps1aH – Cps2J	7%		Cps1bM – Cps2P	40%
	Cps1aH – Cps2M	8%	Hypothetical	Cps1aO – Cps1bN	61%
	Cps1bI – Cps2I	10%	Epimerase	Cps1aP – Cps1bO	93%
	Cps1bI – Cps2J	12%	Aminotransferase	Cps1bF – Cps2G	11%
	Cps1bI – Cps2M	6%		Cps1bF – Cps2K	13%
	Cps1aJ – Cps1bJ	49%	Acetyltransferase	Cps1aI – Cps1bH	12%
	Cps1aJ – Cps2I	9%		Cps1aI – Cps2H	9%
	Cps1aJ – Cps2J	13%		Cps1aI – Cps2L	6%
	Cps1aJ – Cps2M	8%		Cps1aI – Cps2N	10%
	Cps1bJ – Cps2I	8%		Cps1aM – Cps1bH	7%
	Cps1bJ – Cps2J	10%		Cps1aM – Cps2H	7%
	Cps1bJ – Cps2M	9%		Cps1aM – Cps2L	7%
	Cps1aK – Cps1bK	53%		Cps1aM – Cps2N	28%
	Cps1aK – Cps2I	11%		Cps1bH – Cps2H	7%
	Cps1aK – Cps2J	7%		Cps1bH – Cps2L	7%
	Cps1aK – Cps2M	5%		Cps1bH – Cps2N	8%
	Cps1bK – Cps2I	5%			
	Cps1bK – Cps2J	7%			
	Cps1bK – Cps2M	10%			

sequences of the genes in the serotype-specific regions is shown in Table 5. In many genes the homology values between subserotype Ia and Ib/Ic were higher than that between subserotype Ia and serotype II or between subserotypes Ib/Ic and serotype II.

PCR scanning of cps *loci*

Expected sizes of amplification products listed in Table 2 were obtained in 182 *S. parauberis* strains except three strains belonged to subserotype Ic (data not shown), suggesting all the strains possess the same genetic structure of *cps* loci as representative strains of respective serological phenotypes. In the three subserotype Ic strains, one of PCR products was longer than that obtained in NUF1071. By sequencing and analyzing the PCR products, it was revealed that an *ISSdy1*-like element (GenBank accession no., AJ250837) was inserted in *cps1bN* (data not shown).

Discussion

Many bacterial pathogens have a number of antigenic variants due to differences in the polysaccharides present at their cell surface. In streptococci, the cell-surface polysaccharides are most likely known as capsular polysaccharides (CPS). The biosynthesis of CPS always needs some essential enzymes with a complex pathway. The CPS of *Streptococcus pneumoniae* has been well studied and are known to be generally synthesized by the Wzy-dependent pathway (Bentley *et al*., 2006; Yother, 2011). In this study, the genes in the *cps* loci were deduced to encode the initial glycosyltransferase (Undecaprenyl-phosphate galactose phosphotransferase), Wzy polymerase and Wzx flippase, which were needed for the Wzy-dependent pathway. Capsule biosynthesis may be performed as followings: an initial galactose is linked as a galactose phosphate to a membrane-associated lipid carrier (undecaprenyl phosphate) by the initial glycosyltransferase to form the Und-P-P-Gal, to which glycosyltransferases link further monosaccharides sequentially to generate repeat units. Then, Wzx flippase transports the repeat units to the outer surface of the cytoplasmic membrane, and each repeat unit is polymerized to form the lipid-linked CPS by Wzy polymerase. Finally, mature CPS is translocated to the peptidoglycan by the membrane protein complex (Bentley *et al*., 2006).

The genes involved in CPS biosynthesis of *S. parauberis* comprised a gene cluster of cassette-like structure, i.e., conserved genes flanked serotype-specific genes, which is also found in other streptococcal species, such as *S. pneumoniae*, *S. agalactiae* and *S. thermophilus* (Wessels, 1997). *lysR* encoding a transcriptional regulator that belongs to the type 2 periplasmic binding fold protein superfamily can control the synthesis of virulence factors and may play the regula-

tory role in the CPS biosynthetic operon. CpsA is similar to LytR of *Bacillus subtilis* and could be a transcriptional regulator. *cpsA*-deleted mutants of *S. pneumoniae* and *S. agalactiae* have been proved to produce less capsule (Cieslewicz *et al*., 2001). CpsBCD are considered to be a tyrosine kinase phosphoregulatory system which can affect the polymer length. CpsD with the activity of initial phosphorylation requires intracellular interaction with CpsC, while CpsB can block the initial phosphorylation of CpsD, as a kinase inhibitor (Bender and Yother, 2001). The CpsBCD system can be affected by oxygen levels, and reduced aeration conditions were found to correlate with increases in both CpsD phosphorylation levels and capsule production (Weiser *et al*., 2001). *cpsE* identified in *Campylobacter jejuni* encodes sugar-nucleotide-modifying enzyme involved in the biosynthesis of surface carbohydrates (Creuzenet, 2004). It can affect the synthesis of branched chain. In the serotype-specific region, the genes encode the enzymes to construct the repeat units, including an initial glycosyltransferase, additional glycosyltransferases, Wzx flippase, Wzy polymerase and enzymes to modify the repeat units (Yother, 2004). At the lower conserved region, there are two genes, one of which (*cpsQ*) encodes a protein similar to WbpA, an enzyme involved in the biosynthesis of unusual di-N-acetyl-d-mannosaminuronic acid-derived sugar nucleotides found in the O antigen of *Pseudomonas aeruginosa* PAO1 (Miller *et al*., 2004), while the other one encodes hypothetical protein.

The result of PCR scanning suggests that the *cps* locus of *S. parauberis* is generally conserved among different strains of the respective serotypes. The G+C contents of *cps* loci (31.07%–31.51%) were lower than those of whole-genome (35.48%–35.60%) (Nho *et al*., 2013) indicated that the genes of *cps* loci may be imported from other sources. With comparing the structure of the *cps* locus, the distinct features of the *cps* locus were observed in serologically different phenotypes except subserotypes Ib and Ic. Between subserotypes Ib and Ic and between nontypeable strains and serotypes Ib/II, there are several single-base substitution, which may lead to the change of amino acid or the appearance of earlier stop codon, and insertion events in the case of subserotype Ic strains. They could affect the biosynthesis and structure of CPS and lead to reflect in the generation of nontypeable phenotype and subserotypes (Wang *et al*., 2011; Lakkitjaroen *et al*., 2014). High similarities of genes in the serotype-specific region of subserotypes Ia and Ib/Ic could participate in construction of common CPS structure, which resulted in antigenic cross reactivity between them (Kanai *et al*., 2015).

In this study we demonstrated the three kinds of serotype-associated *cps* locus in *S. parauberis* and discussed the CPS biosynthesis pathway and the function of most *cps* genes. In order to better understand the mechanism of diversity arisen among the serological phenotypes of this pathogen, studies on the CPS structure and activity of each gene product will be necessary by biochemical and genetic research such as mutant construction. In this regard, the present works will be an important base for further studies. On the other hand, current works may also contribute to the development of a novel molecular method which can diagnose serotypes rapidly and less expensively.

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