A wide analysis of fish bacterial pathogen *Streptococcus parauberis:* Approach to complementary genomics and proteomics

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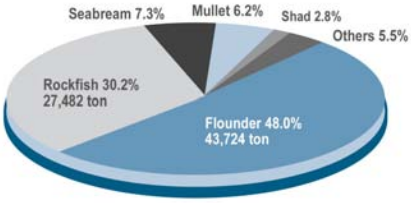
Olive flounder (*Paralichthys olivaceus*)

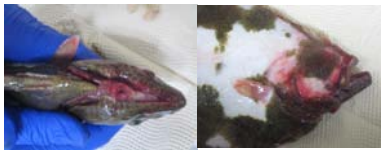


2008 year, Korea

Family: ***Paralichthyidae***

Major etiological agent of olive flounder

***S. parauberis* for streptococcosis of olive flounder (Nho *et al.*, 2009)**



Hemorrhagic septicemia

Exophtalmia

Aims of this study

- To identify and classify *S. parauberis* whole genome
- To gain more knowledge on the antigenic proteins in *S. parauberis* on the molecular genetic level
- To develop vaccine candidates for fish streptococcosis

Complete genome sequence of *S. parauberis*

Experimental Procedures

- **Bacterial strain**

  - *Streptococcus parauberis* KCTC 11537BP (Nho et al., 2009)

- **Preparation of genomic DNA**

  - Genomic DNA was extracted using the QIAGEN Genomic-tip 500/G (Qiagen) and Genomic DNA Buffer set (Qiagen)

- **Genome Sequencer-FLX systems**

DNA library preparation ➢emPCR ➢ sequencing ➢ data analysis

- **Construction of fosmid library**

  - Paired-end sequences in the whole genome sequence assembly provide anchoring information

Assemble results

An ITEM	*ANALYSIS*
GS-FLX titanium run	
Total read No.	192,571
Total length (bp)	75,170,197 bp
Contig No.	181
Fosmid end-sequencing	
Picked clone No.	576
Fosmid read No.	1,146
Fosmid length (bp)	1,021,933 bp
Contig No.	50
Scaffold	
Scaffold No.	6
After Gap-filling	
Designed primers	210
Final Scaffold No.	3

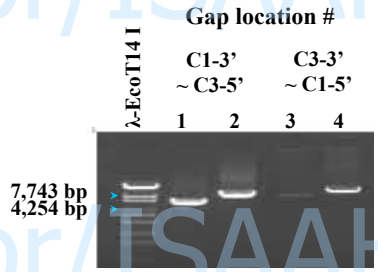
Final 3 scaffold

SEA17.C1 (1,651,649 bps)

SEA17.C2 (3,285 bps)

SEA17.C3 (500,109 bps)

PCR methods for linking



❖ SEA17.C2 was not amplified by any primer sets → SEA17.C2 could not link to SEA17.C1 and SEA 17.C3

❖ SEA17.C1 and SEA17.C3 amplified by primer sets, but could not be sequenced

Primer set #		
1	Forward	G0022_SEA16_PCR_4K-F
	Reverse	G0022_SEA16_PCR_4K-R
2	Forward	G0022_SEA16_PCR_5K-F
	Reverse	G0022_SEA16_PCR_5K-R
3	Forward	G0003_SEA16_PCR_5K-F
	Reverse	G0003_SEA16_PCR_5K-R
4	Forward	G0003_SEA16_PCR_6K-F
	Reverse	G0003_SEA16_PCR_6K-R

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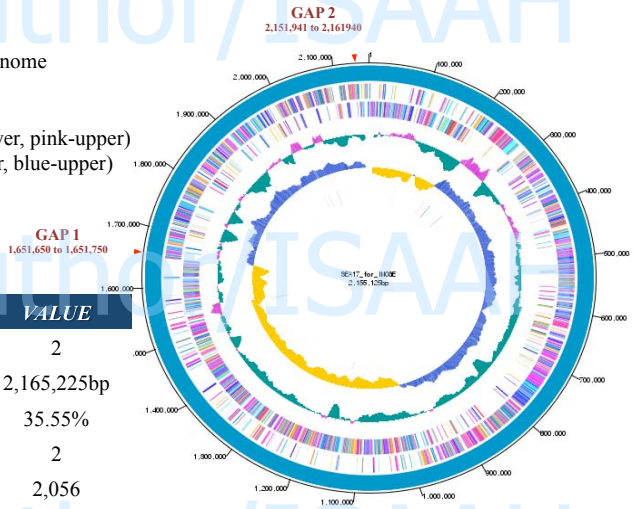
Feature map of *S. parauberis* whole genome

1st circle: Whole *S. parauberis* genome
2nd circle: Forward CDS
3rd circle: Reverse CDS
4th circle: GC contents (green-lower, pink-upper)
5th circle: GC skew (yellow-lower, blue-upper)
6th circle: tRNA



An ITEM		*VALUE*
Total scaffold No.		2
Whole genome lengths (b)		2,165,225bp
GC contents (%)		35.55%
Gap No.		2
Gene annotation	NCBI (manually)	2,056
	CRITICA	2,189
	glimmer	2,256

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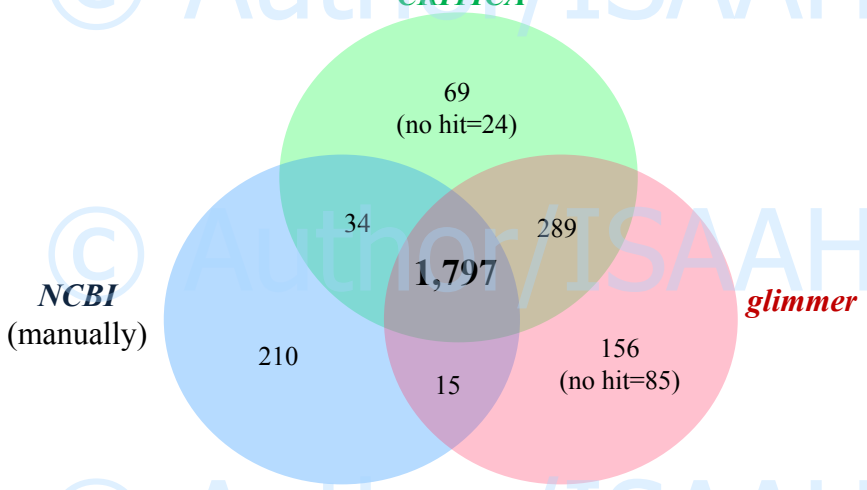
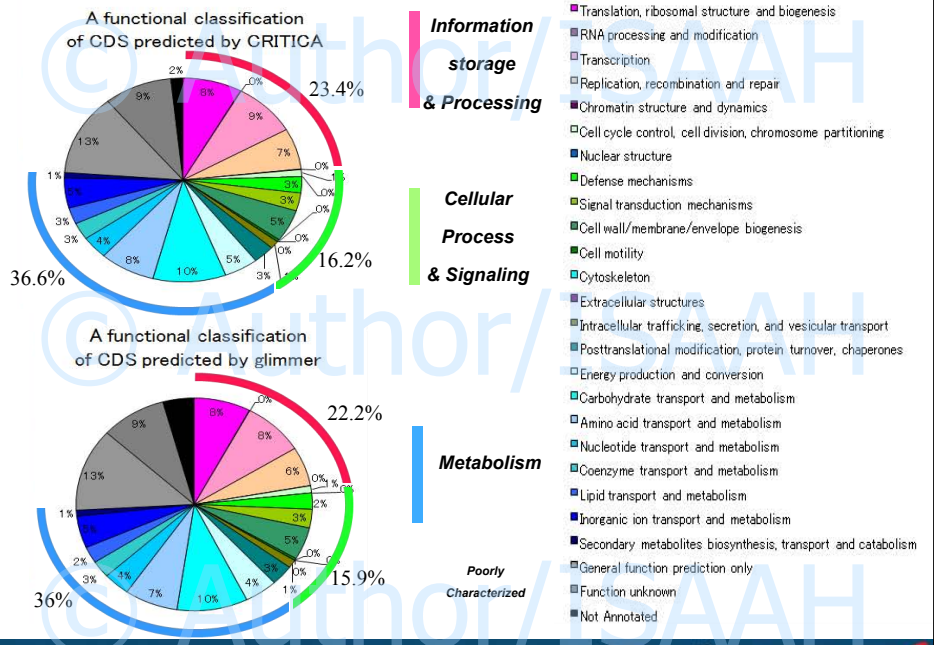
Gene annotation by three methods



❖ 1,797 genes were commonly detected using three annotation methods



Regulation and signaling

No.	Hit-name (locus tag)
1	sigma 54 modulation protein RpoN (SPU0117)
2	RNA polymerase sigma factor RpoD (SPU0910)
3	GTP pyrophosphokinase (SPU1936)
4	ppGpp synthetase (SPU1971)

Energy metabolism

No.	Hit-name (Locus tag)
1	cytochrome d ubiquinol oxidase subunit II (SPU2105)
2	cytochrome d ubiquinol oxidase subunit I (SPU2106)

Protection and environmental survival

No.	Hit-mane (Locus tag)
1	inorganic polyphosphate/ATP-NAD kinase (SPU0754)
2	Polyphosphate kinase (SPU1901)
3	Polyphosphate kinase (SPU1902)

Virulence factor

No.	Hit name (Locus tag)
Fibrinogen binding protein	
1	fibronectin/fibrinogen-binding protein (SPU0147)
2	glyceraldehyde-3-phosphate dehydrogenase (SPU2153)
3	Enolase (SPU0966)
4	SiM protein (SPU2020)
Cell wall sorting signals	
5	sortase A (SPU0724)
6	membrane protein (LPQTG) (SPU1484)
7	lpxtg-motif cell wall anchor domain protein (LPETG) (SPU1866)
8	AMP-dependent synthetase and ligase (LPETG) (SPU2113)
9	alpha-1,2-mannosidase (LPETG) (SPU0134)
10	peptidoglycan biosynthesis protein (LPLTG) (SPU0974)
11	competence protein CoiA-like family protein (LPKTG) (SPU0294)
12	SiM protein (LPSTG) (SPU2020)
13	lysyl-aminopeptidase (LPTTG) (SPU0613)
14	putative cell surface protein (LPTTG) (SPU1607)
15	BBXM (LPVTG) (SPU1618)
16	Xaa-Pro dipeptidyl-peptidase (LPVTG) (SPU1338)
Capsule synthesis	
17	hyaluronate lyase (SPU0191)
18	hyaluronan synthase (SPU1652)
19	UDP-glucose 6-dehydrogenase 2 (SPU0498)
20	UTP--glucose-1-phosphate uridylyltransferase (SPU1657)

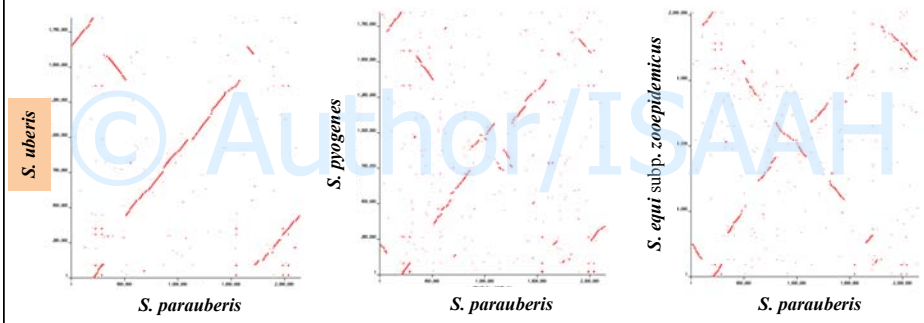
Comparison of *S. parauberis* genome with those of other *Streptococcus* sp.

Homology comparison (whole genome analysis)

S. uberis NC_0120041

S. pyogenes NC_003485.1

S. equi subsp. ***zooepidemicus*** NS_012891

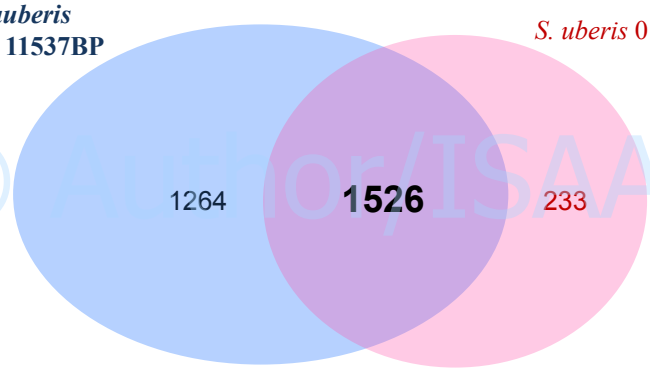


❖ The dot blot assay showed that *S. parauberis* has high homology to *S. uberis*

Consensus genes between S. parauberis and S. uberis (analyzed with whole annotated genes)

S. Parauberis **KCTC 11537BP**

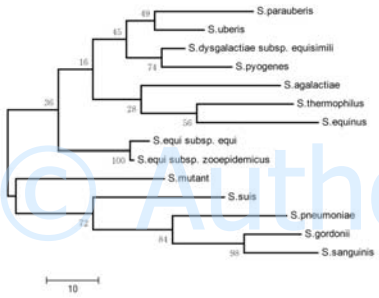
S. uberis 0140J



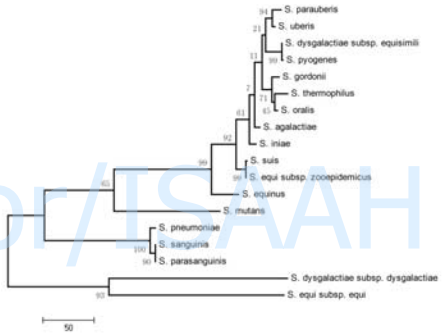
❖ 1,526 *S. parauberis* genes were commonly identified with *S. uberis* with high query coverage

Phylogenic analysis in this study

60kDa chaperonin



GAPDH



*Maximum parsimony with 1,000 times bootstrap replications.

- The 60kDa chaperonin and GAPDH indicate a close relationship between *S. parauberis* and *S. uberis*

2- DE & its Immuno-proteomics

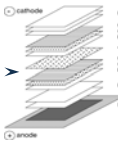
Immunoproteomic procedure

2 weeks

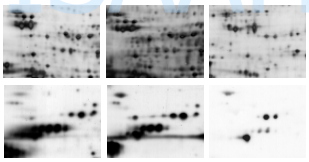
1st Ab: Olive flounder anti- *S. parauberis* serum
2nd Ab: Olive flounder Ig M MAb
3rd Ab: Goat anti-mouse IgG-HRP

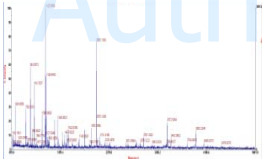
Sample preparation:
S. Parauberis KCTC11537BP

2-DE



Immunoblotting

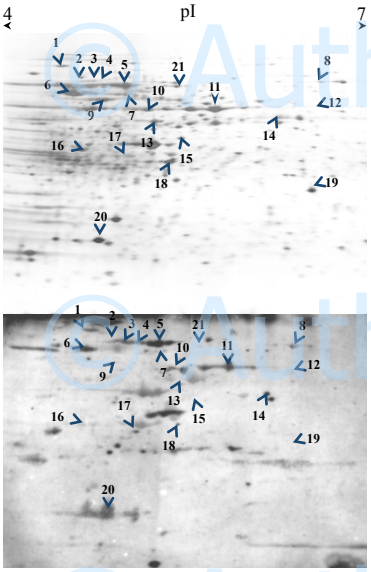




Mass spectrometry-based protein identification

PMF data analysis

❖ This method based on genome data can provide correct proteins



*** Upper image: 2-DE**
*** Lower image: immunoblotting**

No.	Protein name	species	Homology (%)	Function classification
1	Chaperone protein dnaK	*Streptococcus pyogenes* serotype M1	88.20	Posttranslational modification, protein turnover, chaperones
2	60 kDa chaperonin	*Streptococcus agalactiae* serotype Ia	88.03	Posttranslational modification, protein turnover, chaperones
3	GMP synthase	*Streptococcus thermophilus* LMG 18311	88.83	Nucleotide transport and metabolism
4	Galactose-6-phosphate isomerase subunit lacB	*Staphylococcus aureus* subsp. aureus Mu3	84.30	Carbohydrate transport and metabolism
5	Elongation factor Tu	*Streptococcus pyogenes* serotype M1	96.49	Translation, ribosomal structure and biogenesis
6	Enolase	*Streptococcus agalactiae* serogroup Ia	97.02	Carbohydrate transport and metabolism
7	Arginine deiminase	*Streptococcus agalactiae* serogroup Ia	91.02	Amino acid transport and metabolism
8	inosine monophosphate dehydrogenase	*Streptococcus mutans* NN2025	93.12	Nucleotide transport and metabolism
9	phosphoglycerate kinase	*Streptococcus uberis* 0140J	45.10	Carbohydrate transport and metabolism
10	Ornithine carbamoyltransferase, catabolic	*Streptococcus agalactiae* serogroup III	93.20	Amino acid transport and metabolism
11	Glyceraldehyde-3-phosphate dehydrogenase	*Streptococcus dysgalactiae* subsp. *equisimilis*	86.35	Carbohydrate transport and metabolism
12	catabolite control protein A	*Streptococcus pyogenes* MGAS8232	74.25	Transcription
13	L-lactate dehydrogenase	*Streptococcus pyogenes* serotype M1	94.82	Energy production and conversion
14	6-phosphofructokinase	*Streptococcus equi* subsp. *equi* 4047	61.83	Carbohydrate transport and metabolism
15	30S ribosomal protein S2	*Streptococcus agalactiae* serogroup III	93.13	Translation, ribosomal structure and biogenesis
16	Tyrosyl-tRNA synthetase	*Streptococcus pneumoniae* D39	81.86	Translation, ribosomal structure and biogenesis
17	50S ribosomal protein L1	*Streptococcus gordonii* str. Challis	87.39	Translation, ribosomal structure and biogenesis
18	2,3-bisphosphoglycerate-dependent phosphoglycerate mutase	*Streptococcus pyogenes* serotype M1	88.74	Carbohydrate transport and metabolism
19	Uracil phosphoribosyltransferase	*Streptococcus salivarius*	93.81	Nucleotide transport and metabolism
20	Phosphocarrier protein HPr	*Streptococcus equinus*	96.59	Carbohydrate transport and metabolism
21	NADP-dependent glyceraldehyde-3-phosphate dehydrogenase	*Streptococcus uberis* 0140J	89.71	Carbohydrate transport and metabolism

Conclusion

- The *S. parauberis* genome consists of a two scaffold chromoso me (2,165,225 bp with GC content of 35.55%), 1797 genes we re commonly detected
- The 60kDa chaperonin and GAPDH of *S. parauberis* indicate a close relationship to *S. uberis*
- The immunoproteomics method can be used to identify immun ogenic proteins of *S. parauberis* for vaccine candidates
- The genomics and proteomics alliance can produce almost co mplete and accurate gene catalogues for microbial genomes

Acknowledgment

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Thank you for your attention!

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