Study on Appearance and Mechanism of Drug Resistance in Pathogenic Strains of *Streptococcus parauberis* from Japanese Flounder *Paralichthys olivaceus*

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Contents

ABBREVIATIONS

ABPC	Ampicillin
CP	Chloramphenicol
EM	Erythromycin
KM	Kanamycin
LCM	Lincomycin
IPTG	Isopropyl 1-thio-β-D-galactoside
LB-broth	Luria-Bertani broth
MICs	Minimum inhibitory concentrations
ORF	Open reading frame
OA	Oxolinic acid
OTC	Oxytetracycline
SMMX	Sulfamonomethoxine
Tn	Transposon
TH broth	Todd-Hewitt broth
TMP	Trimethoprim
X-Gal	5-bromo-4-chloro-3-indolyl-β-Dgalactopyranoside

Introduction

With increased development of intensive operations, disease has become a significant hurdle to the profitable culture of fish and shellfish. Aquaculture industries in Japan often suffer from severe diseases. For the treatment of bacterial infections in cultured fish, 25 approved chemotherapeutic agents have been used under the guidance of the Ministry of Agriculture, Forestry and Fisheries (Sano, 1998). Because of the extensive usage of chemotherapeutic agents, the prevalence of drug-resistant fish-pathogenic bacteria has caused economic loss in fish culture industry (Aoki, 1988).

Williams and Collins (1990) proposed a new species, *Streptococcus parauberis*, for the genotype II group of *S. uberis* an etiological agent of bovine mastitis. Doménech *et al*. (1996) was the first to identify *S. parauberis* as a fish pathogen by analyzing of the 16S rRNA of several isolates from turbot *Scophthalmus maximus*. Another group reported that this pathogen caused high economic losses in cultured turbot in Spain (Toranzo *et al*., 1995). Since then, streptococcicosis caused by *S. parauberis* resulted in major losses and limited to the turbot and Japanese flounder *Paralichthys olivaceus* cultured in Spain and Korea, respectively (Toranzo *et al*., 2005; Baeck *et al*., 2006). *S. parauberis* has emerged as one of the main pathogenic species in Japanese flounder in Japan since 2002. Based on a serological investigation, two serotypes, I and II, were proposed in Japanese isolates of *S. parauberis* (Kanai *et al*., 2009).

In Japan, for the control of streptococcal infection in Japanese flounder, the use of tetracyclines is the only way approved by the Ministry of Agriculture, Forestry and Fisheries (http://www.maff.go.jp/j/syouan/suisan/suisan_yobo/pdf/suisan_iyakuhin.pdf). Tetracyclines as broad spectrum agents have been widely used for disease treatment in aquaculture in Japan (Kusuda and Salati, 1993; Sano, 1998). However, the worldwide use of tetracycline in the treatment of infections, as prophylactic agents, or as growth promoters, has facilitated the emergence and spread of acquired resistance (Roberts, 2005). The high frequency in detection of tetracycline-resistant bacteria has been reported among various fish pathogens and mariculture environments (Aoki and Takahashi, 1987; Kusuda and Salati, 1993; Sano, 1998; Nonaka and Suzuki, 2002; Kim *et al.*, 2004; Maki *et al.*, 2008). Occurrence of tetracycline resistance among bacterial species is commonly due to three principal mechanisms; 1) efflux of tetracyclines from cells, 2) ribosomal protection (RP) in which the tetracycline-binding site is allosterically disrupted, 3) presence of tetracycline-inactivating enzymes (Chopra and Roberts, 2001; Roberts, 2005). There are currently approximate 40 different tetracycline resistance determinants. In streptococci, the common tetracycline resistance determinants are encoded by the *tet* genes which are usually found in Gram-positive species. Of these *tet* genes reported, *tet*(K) and *tet*(L) were found to be the genes that code the proteins of efflux pump in cells and *tet*(M), *tet*(O) and *tet*(S) were reported to be responsible for RP (Chopra and Roberts, 2001; Roberts, 2005). Among these *tet* genes, *tet*(M) has the widest host range up to 42 genera. The association of *tet*(M), *int* (integrase) and *xis* (excisionase) genes characterizes the Tn*916* –Tn*1545* family (Clewell *et al.*, 1995).

Transferable drug resistances of fish-pathogenic bacteria have been emerged in multi-drug resistant strains. Aoki and his coworkers have suggested a considerable relationship between the increasing number of resistant strains and the indiscriminate use of chemotherapeutics in fish farms (Aoki, 1988). Drug resistance is commonly attributed to the presence of conjugative plasmids and/or conjugative transoposons that have been regarded to be responsible for the drug resistance of various bacteria (Levy and Marshall, 2004). R plasmids have played an important role in the dissemination of antimicrobial resistance genes in the bacterial population with horizontal gene transfer by conjugation. Tn*916,* which encodes resistance to tetracycline, was first documented as a conjugative transposon detected on the chromosome of *Enterococcus faecalis* DS16 (Franke and Clewell, 1981). Since then, Tn*916* has undergone intensive investigation which introduced its wide host range (Rice, 1998), mechanisms of excision and insertion (Caparon and Scott, 1989), as well as conjugal transferability (Clewell *et al.*, 1995) and complete DNA sequence (Flannagan *et al.*, 1994). According to the current information, Tn*916*-like elements have acquired additional resistance determinants, as well as have been integrated within larger multiple resistance elements (Caillaud *et al.*, 1987; Rice and Carias, 1998). Tn*916*-Tn*1545* family transposons and Tn*916*-like elements are currently disseminated with a broad host range (Roberts, 2005), and most contain *tet*(M) (Rice, 1998).

The occurrence and transferability of drug resistances in *S. parauberis* have not been preformed. It is important to determine the antimicrobial susceptibility of a bacterial pathogen, particularly susceptibility to tetracyclines in the case of *S. parauberis*, for the treatment of diseases. The aim of this study was to characterize the antimicrobial susceptibilities of *S. paraubeirs* strains isolated from Japanese flounder in Japan. The resistant strains found were then screened to confirm the resistance determinants.

In the first chapter, the serological differentiation of the Japanese *S. paraubeirs* isolates were described, and their antimicrobial susceptibilities against nine antimicrobial compounds were also investigated. In the second chapter, based on the results of chapter 1, I detected the antimicrobial resistance genes and transposon-related genes. In the third chapter, I characterized the resistance genes harbored by the serotype I resistance strains. The conjugal transferabilities of plasmids were also analyzed by filter mating assay. In the forth chapter, the characterization of Tn*916*-like element harbored by the serotype II strains was described. Since *tet* genes were observed in conjugative plasmid and transposon, the use of tetracyclines for the control of *S. parauberis* infection in Japanese flounder could result in the selection of the resistant strains and transfer of the genes to the other pathogens of animals including humans, being threat to public health.

Chapter 1

Serological differentiation and antimicrobial susceptibilities of *S. parauberis* strains from Japanese flounder

Streptococcus parauberis, a Gram-positive coccus, is known as a causative agent of mastitis in cows as well as streptococcicosis in turbot *Scophthalmus maximus* and Japanese flounder *Paralichthys olivaceus*. It has recently become one of the most important pathogens of cultured Japanese flounder in the western part of Japan.

In this chapter I investigated the serological nature of *S. parauberis* strains from Japanese flounder. It is important to determine the antimicrobial susceptibility of a bacterial pathogen, particularly susceptibility to tetracyclines in the case of *S. parauberis*, for the treatment of diseases. Since it has not been documented the resistance of *S. parauberis* strains in Japan, the minimum inhibitory concentrations (MICs) of nine antimicrobial agents were determined against the *S. parauberis* strains isolated from Japanese flounder.

Materials and Methods

Bacterial strains and culture media

Sixty-four *S. parauberis* strains isolated from Japanese flounder between 2002 and 2007 at commercial aquaculture sites in the western districts of Japan were used in this study (Table 1-1). *S. parauberis* strains were grown in Todd Hewitt (TH, Difco) broth or agar. Stock cultures were stored at －80℃ in TH broth containing 10 % (v/v) glycerol.

Identification of *S. parauberis* by PCR

Identification of the *S. parauberis* strains was performed by PCR amplification of the partial 23S rDNA with the primer pair: S.para-F (5' TTTCGTCTGAGGCAATGTT G 3') and S.para-R (5' GCTTCATATATCGCTATACT 3') (Mata *et al.*, 2004). PCR reaction mixture for detection of the 23S rDNA of *S. parauberis* contained colony of *S. parauberis*, 0.5 μM of each primer, 5 μL of 10 × *Ex Taq* buffer (Mg^{2+} plus), 4μL of dNTP mixture (0.2 mM each) and 1.25 U of *Ex Taq* DNA polymerase (Hot Start Version, TaKaRa) up to a total volume of 50 μL by adding deionized distilled water. PCR amplification was conducted in a thermal cycler and utilized denaturation for 2 min at 95℃. Then, the PCR mixture was subjected to 30 cycles of denaturation at 95℃ for 1 min, annealing at 55℃ for 1 min and extension at 72℃ for 45 s. A final extension was performed at 72℃ for 10 min. The amplified products were analyzed by electrophoresis on a 0.7% agarose gel and stained with ethidium bromide.

Rabbit antisera and serotyping

One milliliter of suspension containing 50 mg formalin-killed cells (FKC) of *S. parauberis* KRS-02083 (serotype I) or KRS-02109 (serotype II) was emulsified with an equal volume of Freund′s complete adjuvant (Wako) and injected subcutaneously to a Japanese white rabbit twice with a 2-week interval. Two weeks after the second injection 50 mg FKC without adjuvant was injected intraperitoneally to the rabbit. Total blood was obtained when the agglutination titer rose to an appropriate value. Antisera were heated for 30 min at 56℃ and stored at -30℃. Serotyping was carried out by slide agglutination test with anti-*S. parauberis* serotype I and II rabbit antisera.

Determination of minimum inhibitory concentration

Minimum inhibitory concentrations (MICs) of antimicrobial agents were determined by the agar dilution method (Japanese Society of Antimicrobials for Animals, the Committee, 2003) using sensitivity test broth (Nissui, Tokyo, Japan) containing 1.5 % agar. The following nine antimicrobial agents were tested: ampicillin (ABPC), chloramphenicol (CP), erythromycin (EM), kanamycin (KM), lincomycin (LCM), oxolinic acid (OA), oxytetracycline (OTC), sulfamonomethoxine (SMMX) and trimethoprim (TMP). The MIC values were determined after incubation at 28℃ for 20 h. Final concentrations of drugs were from 0.016 to 512 μg/mL for ABPC and EM and from 0.125 to 512 μg/mL for CP, KM, LCM, OA, OTC, SMMX and TMP.

Results

Identification of *S. parauberis* strains

All of the strains gave the expected 718bp-PCR amplification product of 23S rDNA specific to *S.parauberis*.

Agglutination reactions of the strains

Sixty-four *S. parauberis* strains isolated from Japanese flounder were serologically divided into two groups, designated serotype I and II, by agglutinating reactions against two kinds of antiserum used in this study. Of the 64 strains, 44 and 20 were found to belong to serotype I and II, respectively (Table 1-1).

Drug susceptibilities

Fig. 1-1 shows the susceptibilities of the 64 strains of *S. parauberis* to nine antimicrobial agents. The exhibitions of MIC distributions were termed as susceptible, intermediately resistant and highly resistant. The MICs of all strains were shown to be one group for CP, KM and TMP as susceptible, while for OA and SMMX as resistant. *S. parauberis* strains exhibited an intrinsic resistance to OA and SMMX. The MICs of both ABPC and LCM presented two groups, 59 strains were susceptible with MIC values of ≦0.016 to 0.063 μg/mL and 0.5 to 1 μg/mL, and five strains were low resistant with MIC values of 0.5 to 1 μg/mL and 4 μg/mL, respectively.

The MICs of EM ranged from ≦0.063 to 512 μg/mL, and the MICs of OTC ranged from 0.5 to 128 μg/mL, respectively. For EM and OTC, the MICs of the 64 strains presented three groups, that is, 39 strains were susceptible, whereas 20 strains were intermediately resistant and five strains were highly resistant. The highly resistant strains were the ABPC/LCM-low resistant strains mentioned above. The EM/OTC-intermediately resistant group consisted with serotype II strains, while the EM/OTC-highly resistant group consisted with serotype I strains. Additionally, OTC-susceptible strains exhibited two peaks; the strains derived from Shikoku Island (Kagawa and Ehime Prefectures) showed a lower MIC value against OTC (0.5 μg/mL) than those from Kyushu Island (Oita Prefecture) (1 to 2 μg/mL).

Discussion

S. parauberis causes a chronic infection and is associated with large-scale mortality in wild and cultured trout and Japanese flounder (Toranzo *et al.* 1995; Baeck *et al.*, 2006; Kanai *et al.*, 2009). Clinical signs of the diseased Japanese flounder observed in Japanese cases are hemorrhages in mouth, on the operculum and in the trunk muscle as well as gill necrosis, and internal signs which are the distended abdomen with hepatomegaly (Fig1-2). These signs were similar to those of Korean diagnosis except for bilateral exophthalmia. Toranzo *et al.* (1995) reported that all of the *S. parauberis* strains from turbot were serologically homogeneous. In this study, 64 Japanese *S. parauberis* isolates derived from Japanese flounder were divided into two serotypes, type I and II, and it was found that the two types were distributed in the western Japan. There are no reports describing the serological nature of Korean *S. parauberis* isolates.

Hence, it is needed to study the serological relationship between Japanese, European and Korean isolates of *S. parauberis*.

MIC values of susceptible strains against ABPC (≦0.016 to 0.063 μg/mL), EM (≦0.016 to 0.063 μg/mL) and OTC (0.5 to 2 μg/mL) were comparable to those of *S. iniae*, another important pathogen of streptococcosis in Japanese flounder, whose MIC values were reported to be 0.00625 to 0.025 μg/mL, 0.05 μg/mL and 0.39 μg/mL, respectively (Sako, 1993). They not only showed similar MIC values as *S. parauberis* but also exhibit an intrinsic resistance to OA and SMMX. It is interesting that MIC values for the susceptible strains derived from Shikoku Island (Kagawa and Ehime Prefectures) against OTC were somewhat lower than those of Kyushu Island (Oita Prefecture). These differences in susceptibility to OTC may reflect variations in the history of tetracycline consumption in each geographical area.

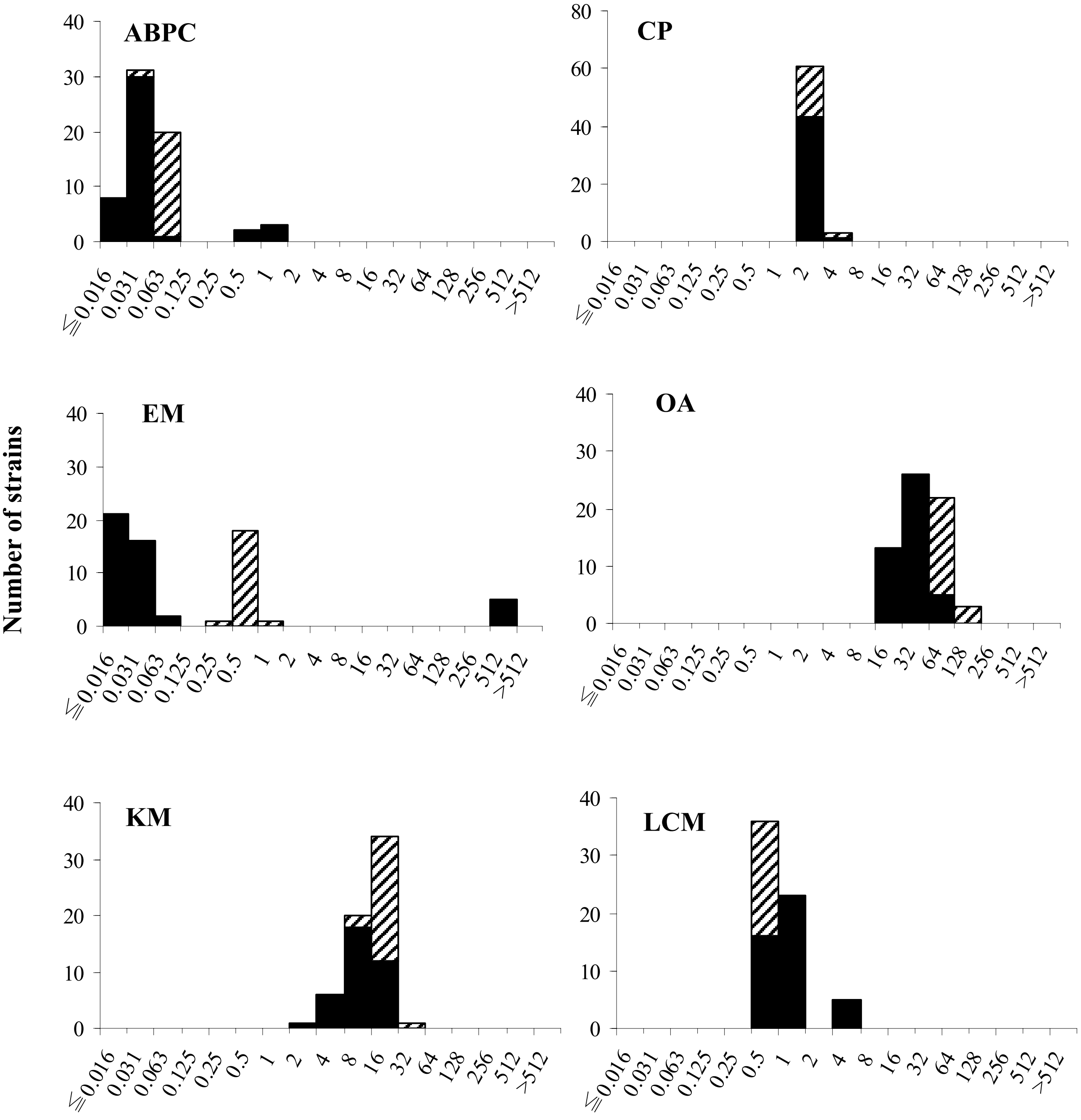
There has been only a short history of using OTC to control the streptococcal infections caused by *S. parauberis* in cultured Japanese flounder since the outbreak of this disease in the beginning of 21 century. However, this chapter showed that the resistant strains for EM and OTC, which is the only qualified drug in treating of streptococcal infection Japanese flounder, have spread among *S. parauberis* serotype II strains in different sites. The long-term use of OTCs to control other streptococcal infections may have contributed to higher rates of antimicrobial resistance in *S. parauberis*. But in our study the effectiveness of the common drug, such as ABPC, was proved for *S. parauberis* infection therapy. In the previous studies, *S. iniae* strains from Japanese flounder were observed to be susceptible to EM/OTC (Sako, 1993), and 17.8% and 14.3 % of 370 *Lactococcus gavieae* isolates from yellowtail *Seriola quinqueradiata* were reported to be intermediately or highly resistant to EM and OTC, respectively (Aoki *et al*, 1990). In comparison with them, a serious problem was present in treatment of streptococcal infections caused by serotype II strains, beacuse in the study all of the *S. parauberis* serotype II strains were intermediately resistant to OTC (Fig. 1-1).

Conclusion

1. Japanese *S. parauberis* isolates from Japanese flounder were divided into two serological phenotypes.
2. *S. parauberis* has an instinct for resistance to SMMX and OA.
3. Five strains of serotype I had low resistance to penicillin (0.5 to 1 μg/mL) and LCM (4 μg/mL) as well as highly resistance to EM (512 μg/mL) and OTC (128 μg/mL).
4. All of serotype II strains were EM (0.25 to 1 μg/mL) and OTC (32 μg/mL) intermediately resistant.

Table 1-1. Number of *Streptococcus parauberis* strains used in this study

Prefecture	Serotype	No. of isolate in the year						Total
		2002	2003	2004	2005	2006	2007	
Kagawa	I	5		3	4			12
	II	8						8
Ehime	I			2	17			19
	II							
Oita	I				4	4	5	13
	II		2		3	2	1	8
Kagoshima	I							
	II					1		1
Nagasaki	I							
	II				1	2		3
Total		13	2	5	29	9	6	64



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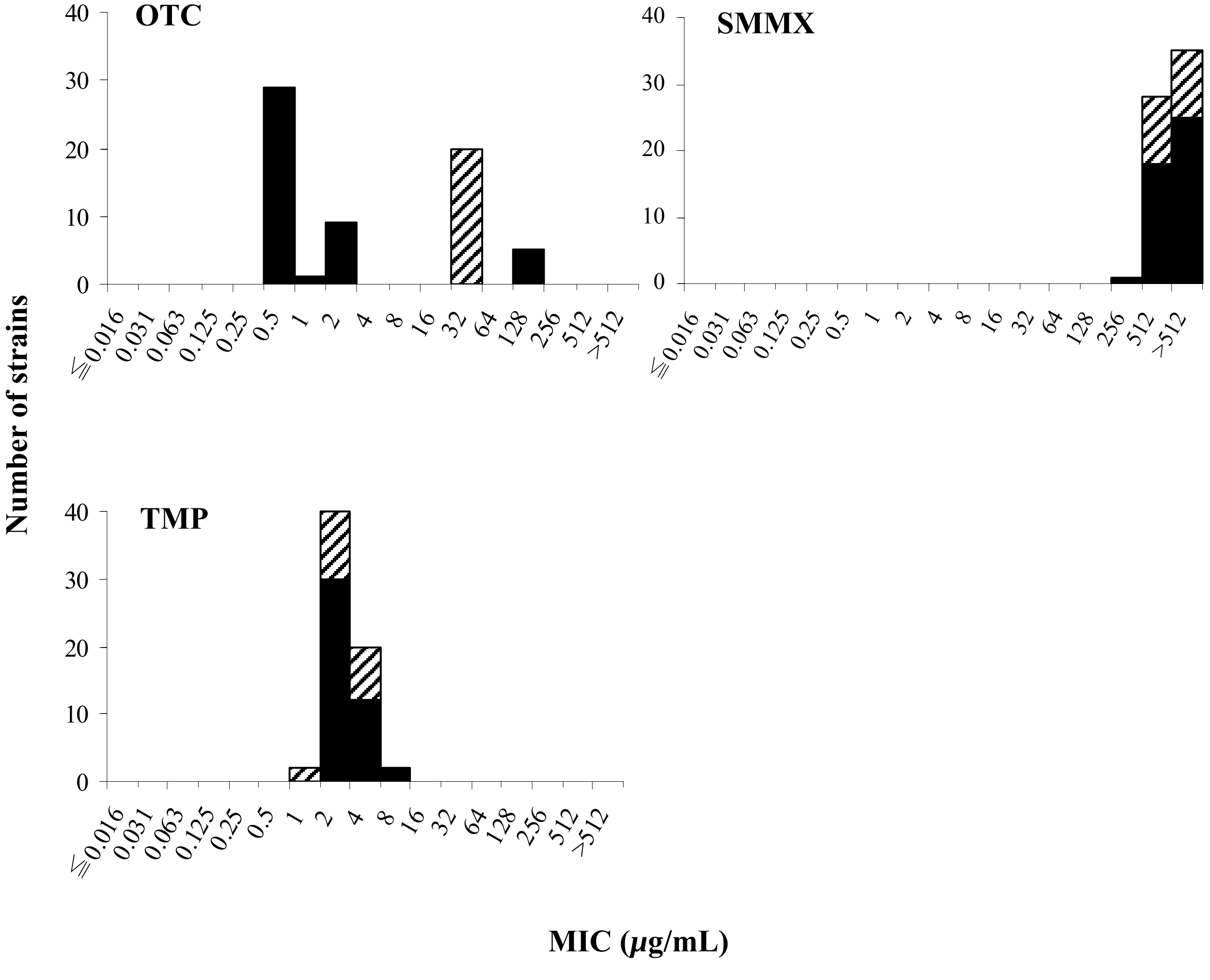

Fig. 1-1. MIC distributions of *Streptococcus parauberis*.

Black bar, *S. parauberis* serotype I strains (n=44); Striped bar, *S. parauberis* serotype II strains (n=20). ABPC, ampicillin; CP, chloramphenicol; EM, erythromycin; KM, kanamycin; LCM, lincomycin; OA, oxolinic acid; OTC, oxytetracycline; SMMX, sulfamonomethoxine; TMP, trimethoprim.

Fig.1-2. Gross pathology of Japanese founder suffering from *Streptococcus parauberis* infection; gill necrosis (A) and hemorrhaging in the trunk muscle (B).

Chapter 2

Screening for antimicrobial resistance and transposon-related genes

In the previous chapter, 5 out of the 44 serotype I strains were highly resistant to EM/OTC, while all of serotype II strains were intermediately resistant to EM/OTC. Comparing with *S. iniae* (Sako, 1993), the relative high proportion of the isolates of *S. parauberis* were found to be resistant to tetracycline (39% of the examined isolates).

As broad-spectrum agents, tetracyclines have been extensively used in human and veterinary chemotherapy. Furthermore, tetracyclines are still used as growth promoters in some countries (Roberts, 2005). Conjugative plasmid and/or transposons encoding drug resistance genes usually play important roles for acquisition of new genes in most bacteria (Roberts, 2005). In multiple antimicrobial resistant streptococci, tetracycline-resistant strains in association with these mobile elements sometimes contain macrolide resistance gene. Some of macrolide resistance genes in streptococci can also occur on other mobile elements, such as conjugative plasmids or member of Tn*916* or Tn*917* family transposons.

In this charpter, the resistance and transposon-related genes were characterized in *S. parauberis* strains.

Materials and methods

Strains, culture and reagents

Five serotype I EM/OTC-resistant strains and all of the serotype II strains (n=20) identified as resistant strains were used for investigation of antimicrobial resistance genes. *Enterococcus faecalis* CG110 containing Tn*916* (Shimoji *et al.*, 1994) was used as a positive control for investigating the presence of Tn*916* family transposons by PCR and Southern blot hybridization. *S. parauberis* and *E. faecalis* strains were grown in Todd-Hewitt broth (THB; Difco). *Escherichia coli* JM109 strain was grown in Luria-Bertani (LB) broth. Drugs were used at the following concentrations: 100 μg/mL for ampicillin, 50μg/mL for X-gal and 0.1mM for IPTG. Chromosomal and plasmid DNA of each strain were prepared using Wizard Genomic DNA Purification Kit (Promega) and QIAprep miniprep kit respectively, according to the manufacturer's protocols.

Screening for antimicrobial resistance and transposon-related genes

Antimicrobial resistance and transposon-related genes were screened by PCR. Oligonucleotide primer sets and PCR condition for detection of each gene are listed in Table 2-1. Macrolide and tetracycline resistance genes were tested on all resistant strains. They were also examined for the presence of *int* (integrase), *xis* (excisionase), *tnpA* (transposase) and *tnpR* (resolvase); the former two genes were associated with conjugative transposons Tn*916*-Tn*1545* family and the latter two were associated with Tn*917* (Shaw and Clewell, 1985). PCR was performed using 50 ng of chromosomal or plasmid DNA as template, 0.5 μM of each primer, 5 μL of 10 × *Ex Taq* buffer (Mg^{2+} plus), 4 μL of dNTP mixture (0.2 mM each) and 1.25 U of *Ex Taq* DNA polymerase (Hot Start Version, TaKaRa) up to a total volume of 50 μL by adding deionized and distilled water. The reaction condition varied according to the primers used and the size of product.

Sequence analysis

Various (nested) PCR products were cloned into the pGEM®-T Easy vector (Promega) and transformed into *E.coli* JM109 Competent Cells (Promega). Plasmid DNA was purified with QIAprep Spin Miniprep Kit (QIAGEN). DNA sequencing was carried out with BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems) and performed on the ABI 3100 DNA sequencer (Applied Biosystems) in Center for Frontier Life Sciences, Nagasaki University. Sequenced data were then assembled and analyzed using DNASIS program (Hitachi Software) and BLAST at the National Center for Biotechnology Information (NCBI) website (www.ncbi.nlm.nih.gov./BLAST). Alignment program ClustalW2 was used for DNA and protein multiple sequence alignment (Larkin *et al.*, 2007).

Results

Detection of genes

erm(A), *erm*(C) or *mef*(A) was not detected in 25 EM-intermediately and highly resistant strains, while the five EM-highly resistant strains gave an expected 224 bp PCR amplification product of *erm*(B) (Fig. 2-1). Tn*917*-related genes, *tnpA* or *tnpR*, were not detected in any of the EM-resistant strains (Table 2-2).

Among 25 OTC-resistant strains, *tet*(M) and *tet*(S) genes were detected in 20 serotype II and five serotype I highly resistant strains, respectively. *int* and *xis* genes of the Tn*916* family were also detected in 20 serotype II strains (Fig. 2-1; Table 2-2). All of the 20 serotype II strains yielded the same PCR reactions for *int* and *xis* genes as *E. faecalis* CG110 strain, the positive control of Tn*916*.

Sequencing analysis

Sequence analysis revealed that *tet*(S) and *erm*(B)gene from *S. parauberis* serotype I shares 99.4% and 99.9% sequence homology with that of plasmid pKL0018 from *Lactococcus garvieae* (accession number AB290882.1). *tet*(M) gene from *S. parauberis* serotype II shares 100% identity to that from *S. agalactiae* (accession number AE009948).

Plasmids detection from serotype I resistant strains

Plasmids about 11 kbp were detected in 5 EM/OTC-resistant strains. PCR products of *tet*(S) were also obtained when plasmids were used as template.

Discussion

Streptococci acquiring OTC resistance commonly according to two mechanisms, including efflux of tetracyclines from cells [coding by *tet*(L) gene] and ribosomal protection [coding by *tet*(M) and *tet*(S) gene] (Roberts, 2005). In this chapter, although *tet*(S) gene was detected in serotype I resistant strains and *tet*(M) gene was detected in all the serotype II strains, both of them shared the same resistant mechanism. Besides *tet*(M) gene, both *int* and *xis* genes were also detected in all the serotype II strains. These genes are known to be components of Tn*916*-Tn*1545* family transposons (Clewell *et al.*, 1995; Chopra and Roberts, 2001). So it is suggested that Tn*916*-related element presents in the *S. parauberis* serotype II strains.

Macrolides are composed of 14 (erythromycin and clarithromycin)-, 15 (azithromycin)-, or 16 (jasamycin, spiramycin, and tylosin)-membered lactones to which amino and/or neutral sugars are attached via glycosidic bonds (Roberts *et al.*, 1999). EM was introduced in 1952 as the first macrolide antibiotic. Unfortunately, within a year, EM-resistant staphylococci from the United States, Europe, and Japan were described (Roberts, 2008). The binding site in the 50S ribosomal subunit for EM overlaps the binding site of the newer macrolides, as well as the structurally unrelated lincosamides and streptogramin B antibiotics. The modification by methylase reduces the binding of all three classes of antibiotics, which results in resistance against macrolids, lincosamides, and streptogramin B antibiotics (MLS). Two distinct mechanisms of macrolide resistance in bacteria have been mainly described, including target site modification of the 23S rRNA by methylation of an adenine residue (MLS phenotype) related to *erm* (designated from erythromycin ribosome methylation) class methylated genes [*erm*(A), (B) and (C)] and efflux pump (M phenotype, resistance to 14- and 15-membered ring macrolide) related to *mef* genes [*mef*(A)] (Roberts, 2008). In this chapter, both *erm*(B) and *tet*(S) genes were detected in 5 serotype I resistant strains and mediated highly resistance to EM and OTC. While *tet*(M) gene was found in all serotype II strains and mediated intermediately resistance to OTC. Additionally, in streptococci, some mobile genetic elements carrying *tet*(M) also harbor macrolide-resistance *erm* genes, such as Tn*1545*, Tn*6002* and Tn*6003* (Fig.2-2) (Cochetti *et al*., 2008). In this study, however, the detection of EM-resistant genes in *S. parauberis* serotype II strains was failed. So these strains may have different mechanisms for EM resistance.

Both the MIC values and types of resistance genes detected suggested a possible association between the serotype and the level of the resistance to EM and OTC. Domelier *et al.* (2008) mentioned that the differences in the bacterial capsule and/or cell wall composition between geno-groups could result in different degrees of susceptibility to transformation or transposition involved in the horizontal transfers of erythromycin resistance genes. Such a mechanism could be applicable to the acquisition of EM and OTC resistance in two types of *S. parauberis*.

Conclusion

1. *tet*(S) and *erm*(B) genes were detected among five *S. parauberis* serotypt I resistant strains.
2. Plasmids that contained *tet*(S) gene were observed in those five *S. parauberis* serotypt I strains.
3. *tet*(M) gene was detected among all of *S. parauberis* serotype II strains.
4. *int* and *xis* genes relating with Tn*916* family were also detected among all of *S. parauberis* serotypt II strains, suggesting the existence of Tn*916*-related element.

Table 2-1. Primers used for PCR detection of genes

Target gene	Primer sequences (5′ to 3′)	PCR production size (bp)	Annealing temperature (℃)	Reference
erm(A)	GTTCAAGAACAATCAATACAGAG GGATCAGGAAAAGGACATTTTAC	420	52	Lina *et al.*, 1999
erm(B)	GTAAACAGTTGACGATATTCTCG CGTACCTTGGATATTCACCG	224*	50	Nagai *et al.*, 2001
erm(C)	GCTAATATTGTTTAAATCGTCAATTCC GGATCAGGAAAAGGACATTTTAC	359	52	Lina *et al.*, 1999
mef(A)	AGTATCATTAATCACTAGTGC TTCTTCTGGTACTAAAAGTGG	345	52	Sutcliffe *et al.*, 1996
tet(K)	TATTTTGGCTTTGTATTCTTTCAT GCTATACCTGTTCCCTCTGATAA	1,159	50	Giovanetti *et al.*, 2003
tet(L)	ATAAATTGTTTCGGGTCGGTAAT AACCAGCCAACTAATGACAATGAT	1,077	50	Giovanetti *et al.*, 2003
tet(M)	GTTAAATAGTGTTCTTGGAG CTAAGATATGGCTCTAACAA	657*	57	Aarestrup *et al.*, 2000
tet(O)	AACTTAGGCATTCTGGCTCAC TCCCACTGTTCCATATCGTCA	515	55	Jeric *et al.*, 2002
tet(S)	ATCAAGATATTAAGGAC TTCTCTATGTGGTAATC	573*	55	Charpentier *et al.*, 1993
int	TGACACTCTGCCAGCTTTAC CCATAGGAACTTGACGTTCG	579	57	Barbeyrac *et al.*, 1996
xis	AGCAGACTGACATTCCTA GCGTCCAATGTATCTATAA	193	55	Amezaga *et al.*, 2002
tnpA	GCTTCCATGGGACTCGGGAC GCTCCCAATTAATAGGAGA	2,133	55	Poyart *et al.*, 2000
tnpR	CCAAGGAGCTAAAGAGGTCCC GTCCCGAGTCCCATGGAAGC	1,547	55	Poyart *et al.*, 2000

* Also used to obtain a specific probe for hybridization.

Table 2-2. Detection of gene markers in *Streptococcus parauberis* resistant strains

Serotype	Strain no.	MIC to OTC (μg/mL)	Gene marker												
			erm(A)	*erm*(B)	*erm*(C)	*mef*(A)	*tet*(K)	*tet*(L)	*tet*(M)	*tet*(O)	*tet*(S)	*tnp*R	*tnp*A	*int*	*xis*
Type I	1	128	−	+	−	−	−	−	−	−	+	−	−	−	−
	2	128	−	+	−	−	−	−	−	−	+	−	−	−	−
	3	128	−	+	−	−	−	−	−	−	+	−	−	−	−
	4	128	−	+	−	−	−	−	−	−	+	−	−	−	−
	5	128	−	+	−	−	−	−	−	−	+	−	−	−	−
Type II	1	32	−	−	−	−	−	−	+	−	−	−	−	+	+
	2	32	−	−	−	−	−	−	+	−	−	−	−	+	+
	3	32	−	−	−	−	−	−	+	−	−	−	−	+	+
	4	32	−	−	−	−	−	−	+	−	−	−	−	+	+
	5	32	−	−	−	−	−	−	+	−	−	−	−	+	+
	6	32	−	−	−	−	−	−	+	−	−	−	−	+	+
	7	32	−	−	−	−	−	−	+	−	−	−	−	+	+
	8	32	−	−	−	−	−	−	+	−	−	−	−	+	+
	9	32	−	−	−	−	−	−	+	−	−	−	−	+	+
	10	32	−	−	−	−	−	−	+	−	−	−	−	+	+
	11	32	−	−	−	−	−	−	+	−	−	−	−	+	+
	12	32	−	−	−	−	−	−	+	−	−	−	−	+	+
	13	32	−	−	−	−	−	−	+	−	−	−	−	+	+
	14	32	−	−	−	−	−	−	+	−	−	−	−	+	+
	15	32	−	−	−	−	−	−	+	−	−	−	−	+	+
	16	32	−	−	−	−	−	−	+	−	−	−	−	+	+
	17	32	−	−	−	−	−	−	+	−	−	−	−	+	+
	18	32	−	−	−	−	−	−	+	−	−	−	−	+	+
	19	32	−	−	−	−	−	−	+	−	−	−	−	+	+
	20	32	−	−	−	−	−	−	+	−	−	−	−	+	+

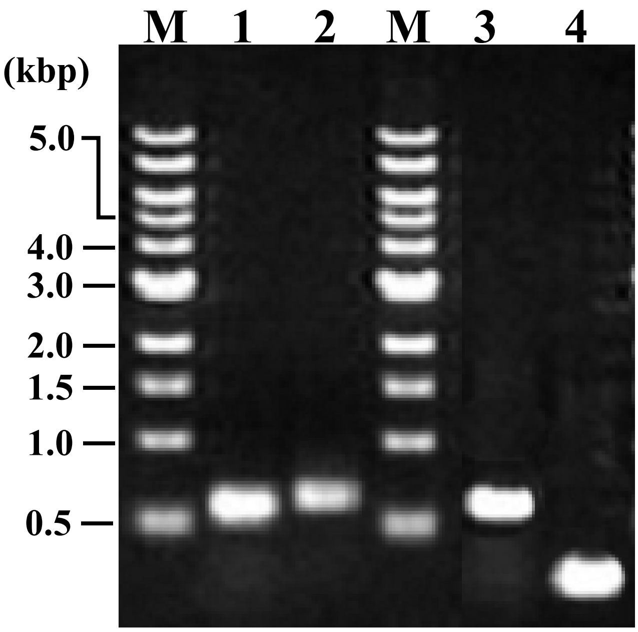

Fig. 2-1. PCR amplifications of resistance genes.

Lane M, DNA size marker (1kbp DNA ladder, New England Biolabs);

Lane 1, *int* PCR product (579 bp); Lane 2, *tet*(M) PCR product (657 bp);

Lane 3, *tet*(S) PCR product (573 bp); Lane 4, *erm*(B) PCR product (224bp).

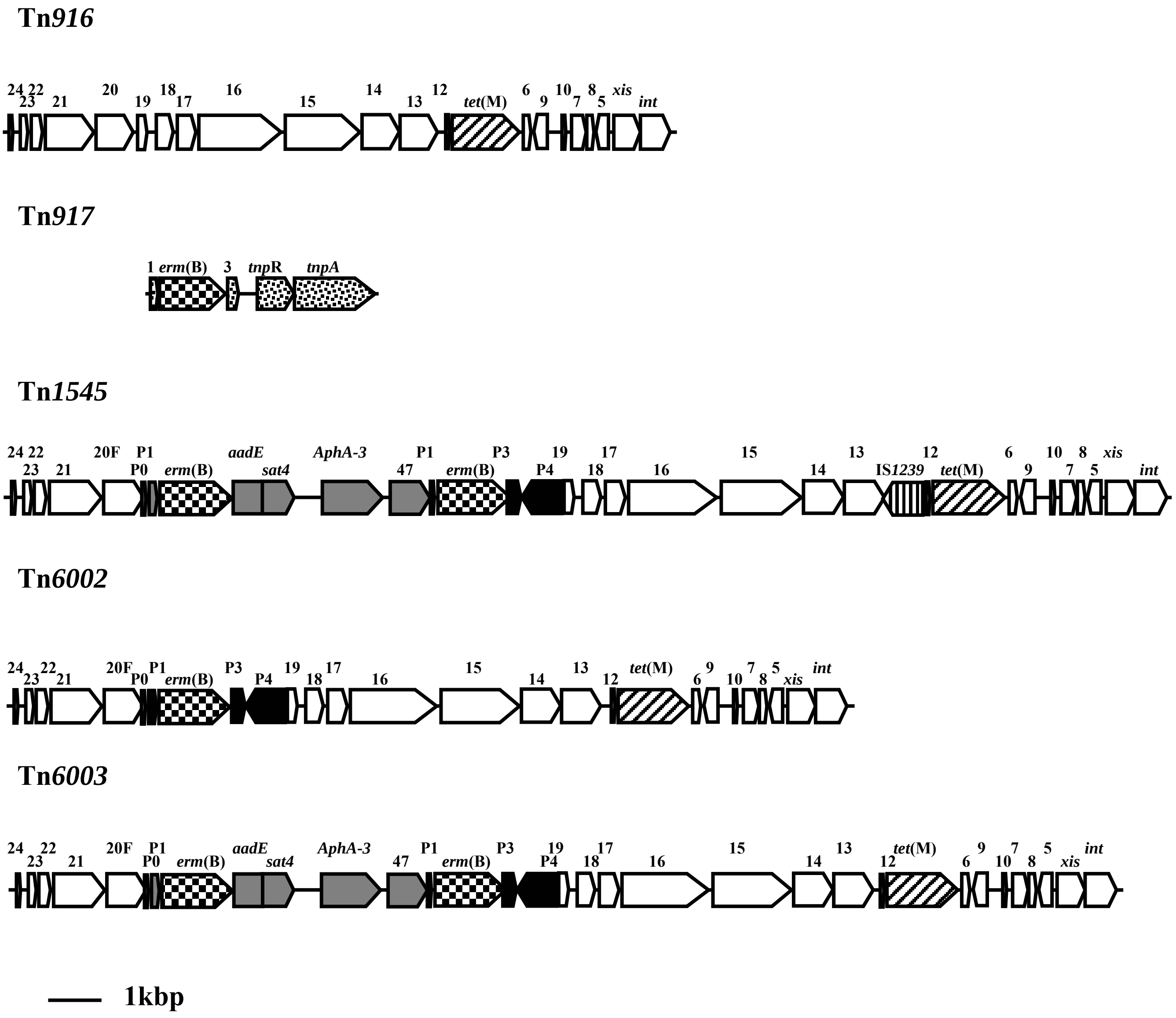

Fig. 2-2. The ORF maps of Tn*917*, Tn*916* and Tn*916* family transposons Tn*6002*, Tn*6003* and Tn*1545*. White arrows indicate Tn*916* ORFs other than *tet*(M) (striped). Spotted arrows indicates the ORFs from Tn*917* other than *erm*(B) (checkered). Black arrows indicated ORFs from the ca. 2.8 kbp *erm*(B) element, except for *erm*(B) (checkered). Light gray arrows indicate ORFs from the ca. 4.2 kbp macrolide-aminoglycoside-streptothricin element from Tn*6003*, except for *erm*(B) (checkered) and a putative IS*1239* transposase (vertical lined).

Chapter 3

Characterization of resistance determinants in *S. parauberis* serotype I strains

In many bacteria, drug resistance is carried on transferable R plasmids. R plasmids have been detected in drug resistant strains of various fish-pathogenic bacteria: *Aeromonas hydrophila, Aeromonas salmonicida, Edwardsiella tarda, Pseudononas fluorescens, Photobacterium damselae* subsp. *piscicida*, *Vibrio anguillarum*, and marine *Vibrio* (Aoki, 1988).

Tetracycline-resistant streptococci are frequently isolated from human pathogens, and resistances are most conferred by genes encoding ribosomal protection proteins (Roberts, 2005). Aoki (1988) reported that the indiscriminate use of antibiotic by fish farmers led to the emergence of drug-resistant strains and could pose a threat to the public health problems, because some streptococcal species are the pathogens of zoonosis. In the previous chapters, the tetracycline resistance conferred by *tet*(S) gene as well as plasmid were detected among the EM/OTC-resistant strains of *S. parauberis* serotype I. *tet*(S) , a member of genes encoding ribosomal protection protein, showed 79% amino acid identity with Tet(M). *tet*(S) gene responsible for plasmid-mediated resistance was initially found on a 37-kbp conjugative plasmid from clinic isolates of *Listeria monocytogenes* (Charpentier *et al.*, 1993). In this chapter, the relationship between the plasmids and resistance genes found in *S. parauberis* serotype I strains, as well as the conjugal transfer of the plasmids, was investigated.

Materials and Methods

Bacterial strains and culture media

Five EM/OTC-highly resistant *S. parauberis* strains were used in this study. *Enterococcus faecalis* FA2-2 (Shiojima *et al.*, 1997) was used as a recipient for conjugation of plasmids. They were grown in Todd-Hewitt broth (THB; Difco). *Escherichia coli* JM109 was grown in Luria-Bertani (LB) broth. Drugs were used at the following concentrations: 100 μg/mL for ampicillin, 50μg/mL for X-gal and 0.1mM for IPTG. Chromosomal and plasmid DNA of each strain were prepared using Wizard Genomic DNA Purification Kit (Promega) and QIAprep miniprep kit, respectively, according to the manufacturer's protocols.

Conjugal transfer of plasmid

Filter mating was carried out by using broth cultures of donor and recipient cells in logarithmic phase. Mixture of the donor and *E. faecalis* FA2-2 cells at a ratio of one donor (50 μL) per 10 recipients (500 μL) was collected on a sterilized membrane filter (0.45 μm, MILLIPORE), and the filter was incubated on a 5% horse blood agar plate at 28℃ overnight. Transconjugants were selected on TH agar plates containing oxytetracycline (30 μg/mL), rifampicin (25 μg/mL) and fusidic acid (25 μg/mL) and incubated at 37℃ overnight. Transfer frequencies were calculated as the number of transconjugants per donor cell. Twenty transconjugants were picked up for testing antibiotic resistance and some of them were used for plasmid DNA purification.

Southern blot hybridization

Based on the results of chapter 2, resistant strains were applied for Southern blot hybridization analysis. Chromosomal DNA (1.5 μg) or plasmid DNA (500 ng) extracted from those strains were completely digested with *Hin*dIII. Digested DNA were resolved by electrophoresis in 0.7 % agarose gel, stained with ethidium bromide and photographed. After depurination, denaturation and neutralization of the gel, DNA was transfered to Hybond N^+ membrane (GE Healthcare) by capillary blotting overnight. The membrane was rinsed in 5×SSC, fixed at 120℃ for 30 min and prehybridized at 55℃ for 30 min in DIG Easy Hyb (Roche). The membrane was then hybridized at 55℃ with DIG-labelled *tet*(S) or *erm*(B) probe (Roche) which was gel-purified PCR product (Table 2-1) labeled with digoxigenin-11-dUTP by random priming using DIG-High Prime (Roche). Washings were under stringent conditions, and signals were detected by exposure of the membrane to Hyperfilm ECL (GE Healthcare).

PCR and Inverse PCR amplification

The primer pairs used in this study are shown in Fig. 3-3. *TaKaRa Ex Taq* Hot start version (Takara Bio) was used for PCR The flanking regions of *erm*(B) were amplified by inverse PCR (Ochman et al. 1988). Briefly, 5μg of genomic DNA was mixed with 12 U of *Hin*dIII in the specified buffer applied by the supplier (Wako, NIPPON GENE, Japan) in a volume of 50 μL and completely digested at 37℃ for 16 h. Digested DNA was extracted twice with phenol equilibrated in TE (pH 8.0) and once with chloroform.

The digested template DNA was precipitated by adding 0.1 volume of 3 M sodium acetate and 2.5 volumes of pre-cold 99% ethanol. The mixture was incubated on ice for 10 min and precipitated DNA was recovered by centrifugation. The precipitated DNA was washed with 200 μL pre-cold 70 % ethanol and dissolved in 30 μL of sterile deionized and distilled water. Ligation mixture comprised 10 μL of cleaved template DNA solution and the equal volume of ligation solution (DNA Ligation Kit, Mighty Mix, TaKaRa). Ligation reaction was then performed at 4℃ for 16h and the ligated DNA was then extracted by phenol and chloroform. The inverse PCR was performed using 2 μL of a serial 10-fold dilution of the circularized DNA, 0.5 μM of each primer, 10× *Ex Taq* buffer (Mg^{2+} plus), 4μL of 0.2mM of dNTP mixture and 1.25U of *Ex Taq* DNA polymerase (Hot Start Version, TaKaRa) up to a total volume of 50μL by adding deionized and distilled water. In order to increase the specificity of the products of inverse PCR, nested PCR was carried out. The PCR was performed on C1000 Thermal Cycler (Bio-Rad).

Sequence analysis

DNA sequencing was carried out as described in the chapter 2.

Results

Conjugal transfer of tetracycline-resistant plasmid

Conjugation experiments were carried out on the five EM/OTC-highly-resistant strains with the plasmid-free recipient strain, *E. faecalis* FA2-2. In transconjugants from each donor, the *tet*(S)-positive plasmid was detected, and its size and *Hin*dIII restriction profile were the same as that in the respective donor strain. From these results it is concluded that the plasmid is a conjugative plasmid. The transfer frequency of the plasmid from each donor to *E. faecalis* FA2-2 was calculated at about 10^{-8} per donor cell by filter mating.

Localization of resistance genes

PCR-amplified products of antimicrobial resistance genes, *tet*(S) and *erm*(B) were used as probes to hybridize with the DNA from resistant strains. Plasmid profiling of the five EM/OTC-highly-resistant strains showed that these strains possessed one plasmid with an approximate size of 11 kbp (data not shown). *Hin*dIII digestion patterns of the plasmids from the five strains were identical, and *tet*(S) was detected on the plasmids (Fig.3-1). The *erm*(B) probe hybridized with the chromosomal DNA of those *tet*(S)-positive strains (Fig. 3-2). Those five strains came from the same prefecture but isolated at different aquaculture sites.

Sequencing analysis of the 2677bp *erm*(B) gene region

The complete nucleotide sequence of the 2677bp *erm*(B) gene region from S. parauberis serotype I strain NUF927 was determined. ORF1 (96bp) is identical to the leader peptide of *erm*(B) gene, whereas ORF2 (738bp) is identical to *erm*(B) gene (Fig. 3-3A). ORF3 (94bp) shows significant homology to the corresponding region in plasmid pKL0018 from *Lactococcus garvieae* (accession number AB290882) and share 100% amino acid identity to those of pKL0018. The known nucleotide sequence (531bp) of ORF4 shares 98.1% identity to that of the ORF298 (531/897bp) in *Clostridium difficile* (accession number AJ294529). A 331bp sequence on the upstream of ORF1 (the *erm*(B) leader peptide) was homologous to another 331bp sequence on the downstream of the ORF3 (Fig. 3-3B). Located downstream of the both 331bp repeat seaquences were 42 and 63bp palindromic sequences, respectively (Fig3-3A). the first palindromic sequence locationg on the upstream, consisted of four direct repeats and two inverted repeats of the 7bp sequence 5'-$^{A}/_{T}$ATCAC$^{A}/_{T}$-3', whereas the second palindromic sequence locating on the downstream, consisted of six direct repeats and 3 inverted repeats (Fig. 3-3A).

Multiple DNA alignment of 2677bp *erm*(B) gene region sequences and phylogenetic analysis

The complete nucleotide sequence of the 2677bp *erm*(B) gene region from *S. parauberis* NUF927 were aligned with those of *Lactococcus lactis* plasmid pI1o8 (accession number AJ549242), *E. faecalis* plasmid pRE25 (accession number X92945), *L. garvieae* plasmid pKL0018 (accession number AB290882), and *Pediococcus acidilactici* plasmid pEOC01 (accession number DQ220741) generated using the ClustalW software(Fig.3-4A). Herein, this fragment revealed strong sequence homology and identity with related regions from other gram-positive bcteria species (Fig.3-4B).

Phylogenetic tree for the 2677bp *erm*(B) gene region shows *S. parauberis* closing as expected with *L. garvieae* (Fig. 3-4C). The *S. parauberis erm*(B) gene region is more similar to *L. garvieae* plasmid pKL0018 than to *Lactococcus lactis* plasmid pI1o8, *E. faecalis* plasmid pRE25 and *Pediococcus acidilactici* plasmid pEOC01.

Discussion

Macrolide antimicrobials are not approved for administrating to Japanese flounder in Japan, but they have been used for treating streptococcicosis causing by *Lactococcus garvieae* in yellowtail *Seriola quinqueradiata* for many years. Several published works showed that *L. garvieae* strains isolated from yellowtail carried transferable R-plasmid on which both *erm*(B) and *tet*(S) were integrated (Aoki *et al.*, 1990; Maki *et al.*, 2008). In addition, *L. garvieae* is also one of the pathogens causing streptococcicosis in cultured Japanese flounder (Fukuda, 2003). Taken together, horizontal spread of resistance genes between streptococci and lactococci might have occurred in aquaculture environment. During preparing this manuscript one serotype I strain of *S. parauberis* which was highly resistant to OTC but susceptible to EM was found in newly collected strains. This strain was isolated in a prefecture different from the prefecture in which above-mentioned plasmids were detected. From the strain one plasmid was detected, the size and *Hin*dIII-digested pattern of which were the same as those of plasmids mentioned above (data not shown). This suggested that the same conjugative plasmid encoding *tet*(S) was conserved and disseminated in the western Japan.

In streptococci, macrolide, licosamide and streptogramin B (MLS) resistance is usually due to an *erm*(B) resistance gene carried by R plasmid or conjugative transposon (Roberts *et al.*, 1999). *erm*(B) gene has been found in numerous bacterial genera, including *Enterococcus*, *Streptococcus*, *Lactobacillus* and *Clostridium* and *Escherichia coli* (Roberts *et al.*, 1999). The results presentd in this study support the hypothesis for the recent horizontal transfer from a *L. garviaea* origin to *S. parauberis* plasmid. Two palindromic sequences comprising of several direct and inverted 7bp repeats with sequence 5'-$^{A}/_{T}$ATCAC$^{A}/_{T}$-3' were identified in the up stream and downstream of *erm*(B) gene. This particular sequence, usually present in R plasmids coding *erm*(B) (e.g. pI1o8, pRE25, pKL0018 and pEOC01) (Fig. 4-4), may have played a role in the insertion of the *erm*(B) gene region into the chromosomal DNA of *S. parauberis* (Cochetti *et al.*, 2007). Some studies have suggested that the similar palindromic sequence may act as hot spots for plasmid cointegrate formation and resolution (Langella *et al.*, 1993).

Analysis of the known amino acid sequence of ORF4 shares 98.1% identity to that of the ORF298 which had similarity to proteins such as δ from pediococcal plasmid pEOC01 (accession number DQ220741), ParA from bacteriophage P1 (accession number YP_006528), pTAR from *Agrobacterium tumefaciens* (accession number AAF45023), RepB from the enterococcal plasmid pAD1 (accession number AAB00504), SopA from the F plasmid (accession number BAA97916), and IncC from plasmid RK2 (accession number BVECIC). These proteins belong to a superfamily of ATPase and have been proposed of demonstrated to be associated with plasmid or chromosomal partitioning. Since the *erm*(B) determinant is located on the nontransferable element in *S. parauberis* serotype I, it is possible that the putative ORF4 (or ORF298) protein may be associated with the partitioning of the original plasmid coding *erm*(B) gene. These results provide evidence that the *erm*(B) gene region may have been transferred from R plasmid.

Further investigations will be carried out to better characterize the *erm*(B) gene region and conjugative plasmid detected in this study and their relevance in *S. parauberis* serotype I epidemiology.

Conclusion

1. *Hin*dIII digestion patterns of the plasmids from the five EM/OTC resistant serotype I strains were identical.
2. *tet*(S) gene was detected in the conjugative plasmid.
3. *erm*(B) probe hybridized with the chromosomal DNA of the strains harboring the conjugative plasmid.
4. The transfer frequency of the conjugative plasmid from each donor to *E. faecalis* FA2-2 was calculated at about 10^{-8} per donor cell by filter mating assay.
5. Two palindromic sequences were comprised of several direct and inverted 7bp repeats with sequence 5'-$^{A}/_{T}$ATCAC$^{A}/_{T}$-3' were identified in the upstream of ORF1 and downstream of ORF3.

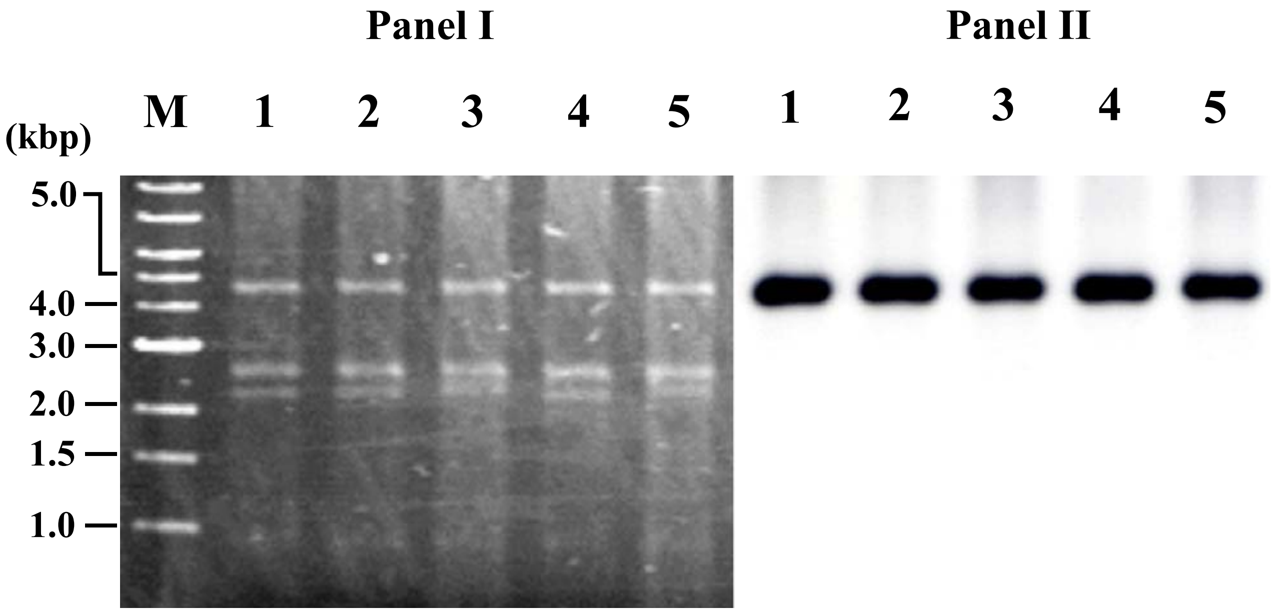

Fig. 3-1. *Hin*dIII-digested plasmid DNAs from EM/OTC highly resistant *S. parauberis* strains (panel I) and Southern blot hybridization analysis of *Hin*dIII-digested plasmid DNAs with *tet*(S) probe (panel II). Lane M, DNA size marker (1kbp DNA ladder, New England Biolabs); Lane 1 to 5, EM/OTC highly resistant *S. parauberis* strains No. 1 to 5.

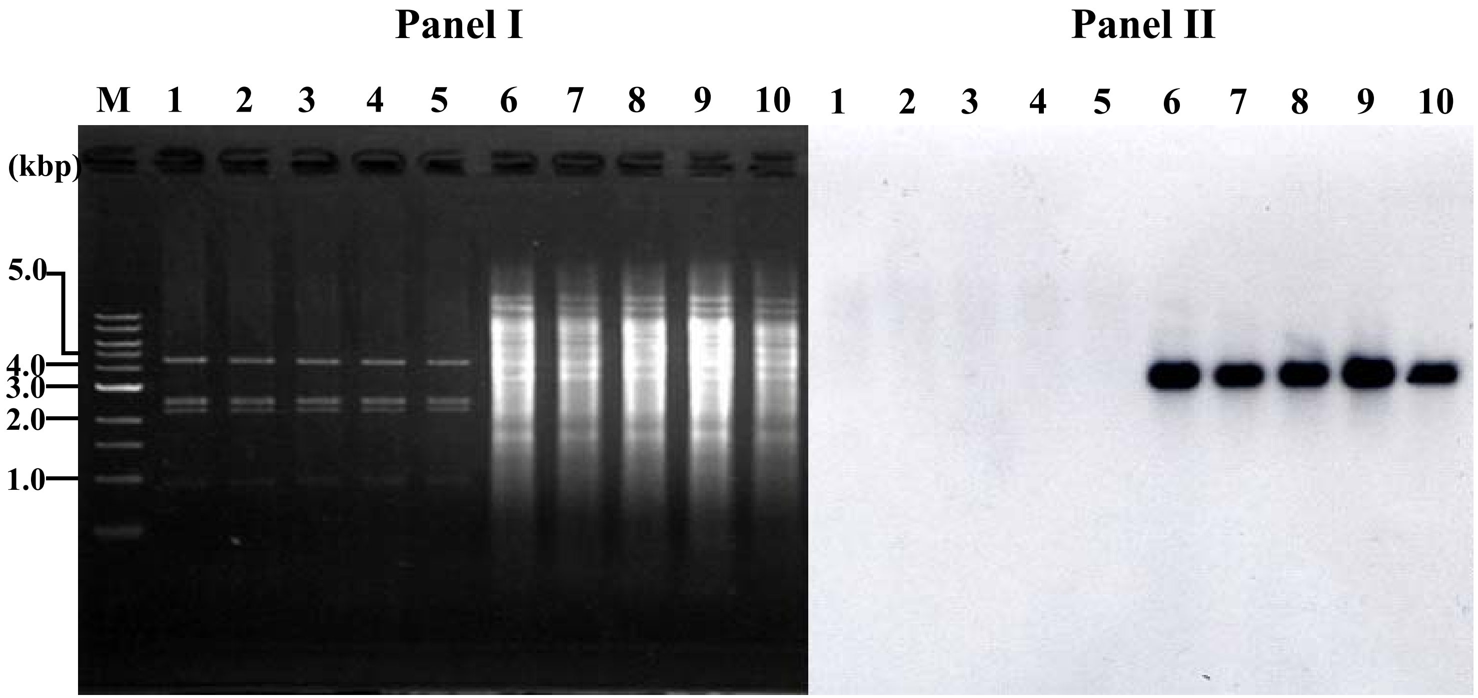

Fig. 3-2. *Hin*dIII-digested plasmid (Lane 1-5) and chromosomal (Lane 6-10) DNAs from EM/OTC highly resistant *S. parauberis* strains (panel I) and Southern blot hybridization analysis of *Hin*dIII-digested plasmid (Lane 1-5) and chromosomal (Lane 6-10) DNAs with *erm*(B) probe (panel II). Lane M, DNA size marker (1kbp DNA ladder, New England Biolabs); Lane 1 to 5 and 6 to10, EM/OTC highly resistant *S. parauberis* strains No. 1 to 5.

A

```
                                                                                          ┌331bp repeat start
   1 ttactggaggctgtccctaccgaatagaaaactaaatttttctgattcccgggaccggaagaaaagaagcagaaaaaagtattgtaggaaaatgtcctaagtgtggcaacaatattgtat  120

 121 taaaaaaatcgttttatggttgttcaaattatcctgaatgtaagtttactttagctgaacattttagaaagaaaaaactcaccaaaacaaatgtaaaagaattactagagggaaaagaa  240

 241 accctggtaaaaggaatcaaaacgaaagatagaaagtcctacaatgccgttgtaaaaatcggagaaaagggatatattgattttatatctttctcaaaataaacataaaagccctttaaa  360

                                       331bp repeat stop┐
 361 gagggcttttatatattaatcacaaatcacttatcacaaatcacaagtgatttgtgattgttgatgataaaataagaataagaagaaatagaaagaagtgagtgattgtgggaaatttag  480
                   →    →    →    →   ←    ←

 481 gcgcacaaaaagaaaaacgaaatgatacaccaatcagtgcaaaaaaagatataatgggagataagacggttcgtgttcgtgctgacttgcaccatatcataaaattttgtataataggaa  600

                                                      ┌ORF1 erm(B) leader peptide start
 601 ttgaagtaaattagatgctaaaatttgtaattaagaaggagggattcgtcatgttggtattccaaatgcgttatcaaatgcgttatgtagataaaacatctactgttttgaacagacta  720
   ORF1 erm(B) leader peptide                         M  L  V  F  Q  M  R  Y  Q  M  R  Y  V  D  K  T  S  T  V  L  K  Q  T  K
                           stop┐
 721 aaaacagtgattacgcagataaataaatacgttagattaattcctaccagtgactaatcttatgactttttaaacagataactaaaattacaaacaaatcgtttaacttctgtatttgtt  840
      N  S  D  Y  A  D  K  .
                                   ┌ORF2 erm(B) start
 841 tatagatgtaatcacttcaggagtgattacatgaacaaaaatataaaatattctcaaaactttttaacgagtgaaaaagtactcaaccaaataataaaacaattgaatttaaaagaaacc  960
   **Primer: IPCRermBF2            M  N  K  N  I  K  Y  S  Q  N  F  L  T  S  E  K  V  L  N  Q  I  I  K  Q  L  N  L  K  E  T
   3′- GGCAAATGCTTTAACCTTG -5′
 961 gataccgtttacgaaattggaacaggtaaagggcatttaacgacgaaactggctaaaataagtaaacaggtaacgtctattgaattagacagtcatctattcaacttatcgtcagaaaaa 1080
     D  T  V  Y  E  I  G  T  G  K  G  H  L  T  T  K  L  A  K  I  S  K  Q  V  T  S  I  E  L  D  S  H  L  F  N  L  S  S  E  K
                                                                                 *Primer:IPCRermBF1 3′- CAACCCTCATAAGGAATGG -5′
1081 ttaaaactgaatactcgtgtcactttaattcaccaagatattctacagtttcaattccctaacaaacagaggtataaaattgttgggagtattccttaccatttaagcacacaaattatt 1200
     L  K  L  N  T  R  V  T  L  I  H  Q  D  I  L  Q  F  Q  F  P  N  K  Q  R  Y  K  I  V  G  S  I  P  Y  H  L  S  T  Q  I  I

1201 aaaaaagtggtttttgaaagccatgcgtctgacatctatctgattgttgaagaaggattctacaagcgtaccttggatattcaccgaacactagggttgctcttgcacactcaagtctcg 1320
     K  K  V  V  F  E  S  H  A  S  D  I  Y  L  I  V  E  E  G  F  Y  K  R  T  L  D  I  H  R  T  L  G  L  L  L  H  T  Q  V  S
                                                                      *Primer:IPCRermBR1 5′- CTTACCCGCCATACCACAG -3′
1321 attcagcaattgcttaagctgccagcggaatgctttcatcctaaaccaaaagtaaacagtgtcttaataaaacttacccgccataccacagatgttccagataaatattggaagctatat 1440
     I  Q  Q  L  L  K  L  P  A  E  C  F  H  P  K  P  K  V  N  S  V  L  I  K  L  T  R  H  T  T  D  V  P  D  K  Y  W  K  L  Y
                                                            **Primer:IPCRermBR2 5′- TCAAGCAATGAAACACGCC -3′
1441 acgtactttgtttcaaaatgggtcaatcgagaatatcgtcaactgtttactaaaaatcagtttcatcaagcaatgaaacacgccaaagtaaacaatttgagtaccgttacttatgagcaa 1560
     T  Y  F  V  S  K  W  V  N  R  E  Y  R  Q  L  F  T  K  N  Q  F  H  Q  A  M  K  H  A  K  V  N  N  L  S  T  V  T  Y  E  Q
                                               ORF2 erm(B) stop┐   ┌ ORF3 start
1561 gtattgtctattttttaatagttatctattatttaacgggaggaaataattctatgagtcgcttttgtaaatttggaaagttacacgttactaaagggaatgtagataaattattaggtat 1680
     V  L  S  I  F  N  S  Y  L  L  F  N  G  R  K  .     M  S  R  F  C  K  F  G  K  L  H  V  T  K  G  N  V  D  K  L  L  G  I
                            ORF3 stop┐                                                                              ┌331bp repeat start
1681 actactgacagcttccaaggagctaaagaggtccctagcgcctacggggaatttgtatcgataaggggtacaaattcccactaagcgctcgggaccccttgtaggaaaatgtcctaagtg 1800
     L  L  T  A  S  K  E  L  .

1801 tggcaacaatattgtattaaaaaaatcgttttatggttgttcaaattatcctgaatgtacsttcactttagctgaacattttagaaagaaaaaactcaccaaaacaaatgtaaaagaatt 1920

1921 actagagggaaaagaaaccctggtaaaaggaatcaaaacgaaagatagaaagtcctacaatgccgttgtaaaaatcggagaaaagggatatattgattttatctctttttcaaaataagc 2040

                                                                    331bp repeat stop┐                        ┌ORF4 start
2041 ataaaagccctttaaagagggcttttatatattaatcacaaatcacttatcacaaatcacaagtgattaatcacaaatcacttgtgatttgtgattcctaatgatacaatattactatac 2160
                                             →    →    →    →   ←    →    →   ←    ←                           M  I  Q  Y  Y  Y  T

2161 aaaaaaagaatggggcgtagttatggagaaggaagaactcaaaatacttgaagaattaagacgtattttaaacagtaaaaatgaagcgattgttatcttaaacaattattttaaaggtgg 2280
      K  K  E  W  G  V  V  M  E  K  E  E  L  K  I  L  E  E  L  R  R  I  L  N  S  K  N  E  A  I  V  I  L  N  N  Y  F  K  G  G

2281 tgttggaaagtcaaaattatcgactatgtttgcttacttgacagacaaatttaatttaaaagttttaatgatcgataaggacttacaagcaacattgacaaaagacttagcaaaaacatt 2400
      V  G  K  S  K  L  S  T  M  F  A  Y  L  T  D  K  F  N  L  K  V  L  M  I  D  K  D  L  Q  A  T  L  T  K  D  L  A  K  T  F

2401 taaggtagaattgccacgtgttaattttatgaaggactgaaaaatggaaacttggcttcttctattgttcatttgactgataatttagacttgatccctggcacgtttgatttgatgtt 2520
      K  V  E  L  P  R  V  N  F  Y  E  G  L  K  N  G  N  L  A  S  S  I  V  H  L  T  D  N  L  D  L  I  P  G  T  F  D  L  M  L

2521 actgccaaaattaactcgctcatggacttttgaaaatgaaagtagattgcttgctactcttttagcacctttaaaaagtgactatgatctcattattatggatactgtaccaccgccaag 2640
      L  P  K  L  T  R  S  W  T  F  E  N  E  S  R  L  L  A  T  L  L  A  P  L  K  S  D  Y  D  L  I  I  M  D  T  V  P  P  P  S

2641 cgtttatccaaataatgcaatcgtggcagtgaatacc                                                                                     2677
      V  Y  P  N  N  A  I  V  A  V  N  T
```

B

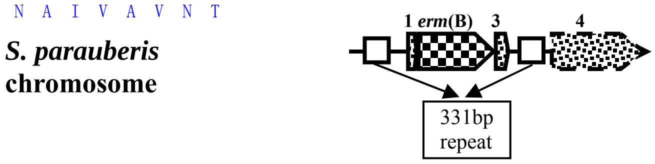

Fig. 3-3. (A) The DNA and amino acid sequences of the 2677bp *erm*(B) gene region from *S. parauberis* serotype I resistance strain (NUF927). The primer sets used for inverse and nested PCRs are also indicated. The grey regions indicate the 311bp repeat sequences. The 7bp repeats and their relative onriertations are indicated by arrows below the nucleotide sequences. Stop codon is indicated by dot. * Primers used for inverse PCR. ** Primers used for nested PCR. (B) Chromosomal location of *S. parauberis erm*(B) resistanc gene. A checkered arrow indicates *erm*(B) and dotted arrows idicates ORFs. A small white box indicates a 331bp repeat on the upstream and downstream of the *erm*(B) gene.

A

```
                                                                                                     ┌331bp repeat start
L. lactis       TTACTGGAAGCTGTACCTAACGATATAGAAAAACTAAATTTTTCTGATTACCAGGAACAGAAAGAAAAAGAAGCAGAAAAAAGTATTGTAGGAAAATGTCCTAAGTGTGGCAACAATATT 120
E. faecalis     TTACTGGAAGCTGTACCTAACGATATAGAAAAACTAAATTTTTCTGATTACCAGGAACAGAAAGAAAAAGAAGCAGAAAAAAGTATTGTAGGAAAATGTCCTAAGTGTGGCAACAATATT 120
L. garvieae     TTACTGGAAGCTGTACCTAACGATATAGAAAAACTAAATTTTTCTGATTACCAGGAACAGAAAGAAAAAGAAGCAGAAAAAAGTATTGTAGGAAAATGTCCTAAGTGTGGCAACAATATT 120
P. acidilactici TTATTGGAAAATGTACCTACCGATATAGAGAAATTAAGTTTTGCTGATTACCAGGAACAGAAAGAAAAAGAAGCAGAAAAAAATATTGTTGGAAAATGTCCTAAGTGTGGCAACAATATT 120
S. parauberis   TTACTGGAGGCTGTCCCTACCGA-ATAGA-AAACTAAATTTTTCTGATTCCCGGGACCGGAAG--AAAAGAAGCAGAAAAAAGTATTGTAGGAAAATGTCCTAAGTGTGGCAACAATATT 116
                *** ****   *** **** *** ***** *** *** **** ****** ** *** * ***   ****************  ****** ******************************

L. lactis       GTATTAAAAAAATCGTTTT-ATGGTTGTTCAAATTATCCTGAATGTAAGTTTACTTTAGCTGAACATTTTAGAAAGAAAAAACTAACCAAAACGAATGTAAAAGAATTACTGGAGGGAAA 239
E. faecalis     GTATTAAAAAAATCGTTTT-ATGGTTGTTCAAATTATCCTGAATGTAAGTTTACTTTAGCTGAACATTTTAGAAAGAAAAAACTAACCAAAACGAATGTAAAAGAATTACTGGAGGGAAA 239
L. garvieae     GTATTAAAAAAATCGTTTT-ATGGTTGTTCAAATTATCCTGAATGTAAGTTTACTTTAGCTGAACATTTTAGAAAGAAAAAACTAACCAAAACGAATGTAAAAGAATTACTGGAGGGAAA 239
P. acidilactici GTATTAAAAAAATCGTTTT-ATGGTTGTTCAAATTATCCTGAATGTAAGTTTACTTTAGCTGAACATTTTAGAAAGAAAAAACTAACCAAAACAAATGTAAAAGAATTACTAGAGGGAAA 239
S. parauberis   GTATTAAAAAAATCGTTTTTATGGTTGTTCAAATTATCCTGAATGTAAGTTTACTTTAGCTGAACATTTTAGAAAGAAAAAACTCACCAAAACAAATGTAAAAGAATTACTAGAGGGAAA 236
                ******************* **************************************************************** ******** ***************** ********

L. lactis       AGAAACCCTGGTAAAAGGAATCAAAAACAAAGAGAAAAAGCCCTACAATGCCGTTGTAAAAATTGGGGAAAAGGGATATATTGATTTTATATCTTTCTCAAAATAAACATAAAAGCCCTT 359
E. faecalis     AGAAACCCTGGTAAAAGGAATCAAAAACAAAGAGAAAAAGCCCTACAATGCCGTTGTAAAAATTGGGGAAAAGGGATATATTGATTTTATATCTTTCTCAAAATAAACATAAAAGCCCTT 359
L. garvieae     AGAAACCCTGGTAAAAGGAATCAAAAACAAAGAGAAAAAGCCCTACAATGCCGTTGTAAAAATTGGGGAAAAGGGATATATTGATTTTATATCTTTCTCAAAATAAACATAAAAGCCCTT 359
P. acidilactici AGAAACCCTGGTAAAAGGAATCAAAACGAAAGATAAAAAGTCCTACAATGCCGTTGTAAAAATTGGAGAAAAGGGATATATTGATTTTATATCTTTCTCAAAATAAACATAAAAGCCCTT 359
S. parauberis   AGAAACCCTGGTAAAAGGAATCAAAACGAAAGATAGAAAGTCCTACAATGCCGTTGTAAAAATCGGAGAAAAGGGATATATTGATTTTATATCTTTCTCAAAATAAACATAAAAGCCCTT 356
                **************************  ***** * **** ********************** ** *****************************************************

                                                                    331bp repeat stop┐
L. lactis       TAAAGAGGGCTTTTATATATTAATCACAAATCACTTATCACAAATCACTTATCACAAATCACAAGTGATTTGTGATTGTTGATGATAAAATAAGAATAAGAAGAAATAGAAAGAAGTGAG 479
E. faecalis     TAAAGAGGGCTTTTATATATTAATCACAAATCACTTATCACAAATCACTTATCACAAATCACAAGTGATTTGTGATTGTTGATGATAAAATAAGAATAAGAAGAAATAGAAAGAAGTGAG 479
L. garvieae     TAAAGAGGGCTTTTATATATTAATCACAAATCACTTATCACAAATCACTTATCACAAATCACAAGTGATTTGTGATTGTTGATGATAAAATAAGAATAAGAAGAAATAGAAAGAAGTGAG 479
P. acidilactici TAAATAGGGCTTTTATATATTAATCACAAATCACTTATCA--------------CAAATCACAAGTGATTTGTGAT----------AAAATAAGAATAAGAAGAAATAGAAAGAAGTGAG 455
S. parauberis   TAAAGAGGGCTTTTATATATTAATCACAAATCACTTATCA--------------CAAATCACAAGTGATTTGTGATTGTTGATGATAAAATAAGAATAAGAAGAAATAGAAAGAAGTGAG 462
                **** ***********************************              **********************          **********************************

L. lactis       TGATTGTGGGAAATTTAGGCGCACAAAAAGAAAAACGAAATGATACACCAATCAGTGCAAAAAAAGATATAATGGGAGATAAGACGGTTCGTGTTCGTGCTGACTTGCACCATATCATAA 599
E. faecalis     TGATTGTGGGAAATTTAGGCGCACAAAAAGAAAAACGAAATGATACACCAATCAGTGCAAAAAAAGATATAATGGGAGATAAGACGGTTCGTGTTCGTGCTGACTTGCACCATATCATAA 599
L. garvieae     TGATTGTGGGAAATTTAGGCGCACAAAAAGAAAAACGAAATGATACACCAATCAGTGCAAAAAAAGATATAATGGGAGATAAGACGGTTCGTGTTCGTGCTGACTTGCACCATATCATAA 599
P. acidilactici AGATCGTGGGAAATTTAGGCGCACAGAAAGCAAAACGAAATGATACGCCAATCAGCGCAAAAAAAGATATAATGGGGGATAAGACGGTTCGTGTTCGTGCTGACTTGCACCATATCATAA 575
S. parauberis   TGATTGTGGGAAATTTAGGCGCACAAAAAGAAAAACGAAATGATACACCAATCAGTGCAAAAAAAGATATAATGGGAGATAAGACGGTTCGTGTTCGTGCTGACTTGCACCATATCATAA 582
                 *** ******************** **** *************** ******** ******************** *******************************************

L. lactis       AAATCGAAACAGCAAAGAATGGCGGAAACGTAAAAGAAGTTATGGAAATAAGACTTAGAAGCAAACTTAAGAGTGTGTTGATAGTGCATTATCTTAAAATTTTGT--ATAATAGGAATTG 717
E. faecalis     AAATCGAAACAGCAAAGAATGGCGGAAACGTAAAAGAAGTTATGGAAATAAGACTTAGAAGCAAACTTAAGAGTGTGTTGATAGTGCATTATCTTAAAATTTTGT--ATAATAGGAATTG 717
L. garvieae     AAATCGAAACAGCAAAGAATGGCGGAAACGTAAAAGAAGTTATGGAAATAAGACTTAGAAGCAAACTTAAGAGTGTGTTGATAGTGCAGTATCTTAAAATTTTGTGTATAATAGGAATTG 719
P. acidilactici AAATCGAAACAGCAAAGAATGGCGGAAACGTAAAAGAAGTTATGGAAATAAGACTTAGAAGCAAACTTAAGAGTGTGTTGATAGTGCAGTATCTTAAAATTTTGT--ATAATAGGAATTG 693
S. parauberis   AA-------------------------------------------------------------------------------------------------TTTTGT--ATAATAGGAATTG 603
                **                                                                                                 ******  *************

                                                                 ┌ORF1 erm(B) leader peptide start
L. lactis       AAGTTAAATTAGATGCTAAAAATTTGTAATTAAGAAGGAGGGATTCGTCATGTTGGTATTCCAAATGCGTAAT------------GTAGATAAAACATCTACTGTTTTGAAACAGACTAA 825
E. faecalis     AAGTTAAATTAGATGCTAAAAATTTGTAATTAAGAAGGAGGGATTCGTCATGTTGGTATTCCAAATGCGTAAT------------GTAGATAAAACATCTACTGTTTTGAAACAGACTAA 825
L. garvieae     AAGTTAAATTAGATGCTAAAAATTTGTAATTAAGAAGGAGGGATTCGTCATGTTGGTATTCCAAATGCGTAAT------------GTAGATAAAACATCTACTGTTTTGAAACAGACTAA 827
P. acidilactici AAGTTAAATTAGATGCTAAAAATTTGTAATTAAGAAGGAGGGATTCGTCATGTTGGTATTCCAAATGCGTTATCAAATGCGTTATGTAGATAAAACATCTACTGTTTTGAAACAGACTAA 813
S. parauberis   AAGT-AAATTAGATGCTAAAA-TTTGTAATTAAGAAGGAGGGATTCGTCATGTTGGTATTCCAAATGCGTTATCAAATGCGTTATGTAGATAAAACATCTACTGTTTTGAAACAGACTAA 721
                **** **************** ************************************************ **            ***********************************

        ORF1 erm(B) leader peptide  stop┐
L. lactis       AAACAGTGATTACGCAGATAAATAAATACGTTAGATTAATTCCTACCAGTGACTAATATTATGACTTTTTAAACAGATAACTAAAATTACAAACAAATCGTTTAACTTCTGTATTTGTTT 945
E. faecalis     AAACAGTGATTACGCAGATAAATAAATACGTTAGATTAATTCCTACCAGTGACTAATCTTATGACTTTTTAAACAGATAACTAAAATTACAAACAAATCGTTTAACTTCTGTATTTGTTT 945
L. garvieae     AAACAGTGATTACGCAGATAAATAAATACGTTAGATTAATTCCTACCAGTGACTAATCTTATGACTTTTTAAACAGATAACTAAAATTACAAACAAATCGTTTAACTTCTGTATTTATTT 947
P. acidilactici AAACAGTGATTACGCAGATAAATAAATACGTTAGATTAATTCCTACCAGTGACTAATCTTATGACTTTTTAAACAGATAACTAAAATTACAAACAAATCGTTTAACTTCTGTATTTGTTT 933
S. parauberis   AAACAGTGATTACGCAGATAAATAAATACGTTAGATTAATTCCTACCAGTGACTAATCTTATGACTTTTTAAACAGATAACTAAAATTACAAACAAATCGTTTAACTTCTGTATTTGTTT 841
                ******************************************************** *********************************************************** ***

                                             ┌ORF2 erm(B) start
L. lactis       ATAGATGTAATCAATTCAGGAGAGATTACATGAACCAAAATATAAAATATTCTCAAAACTTTTTAACGAGTGAAAAAGTACTCAACCAAATAATAAAACAATTGAATTTAAAAGAAACCG 1065
E. faecalis     ATAGATGTAATCACTTCAGGAGAGATTACATGAACAAAAATATAAAATATTCTCAAAACTTTTTAACGAGTGAAAAAGTACTCAACCAAATAATAAAACAATTGAATTTAAAAGAAACCG 1065
L. garvieae     ACAGATGTAATCACTTCAGGAGTGATTACATGAACAAAAATATAAAATATTCTCAAAACTTTTTAACGAGTGAAAAAGTACTCAACCAAATAATAAAACAATTGAATTTAAAAGAAACCG 1067
P. acidilactici ATAGATGTAATCACTTCAGGAGTGATAACATGAACAAAAATATAAAATATTCTCAAAACTTTTTAACGAGTGAAAAAGTACTCAACCAAATAATAAAACAATTGAATTTAAAAGAAACCG 1053
S. parauberis   ATAGATGTAATCACTTCAGGAGTGATTACATGAACAAAAATATAAAATATTCTCAAAACTTTTTAACGAGTGAAAAAGTACTCAACCAAATAATAAAACAATTGAATTTAAAAGAAACCG 961
                * *********** ******** *** ******** ************************************************************************************

L. lactis       ATACCGTTTACGAAATTGGAACAGGTAAAGGGCATTTAACGACGAAACTGGCTAAAATAAGTAAACAGGTAA---CTATTGAATTAGACAGTCATCTATTCAACTTATCGTCAGAAAAAT 1182
E. faecalis     ATACCGTTTACGAAATTGGAACAGGTAAAGGGCATTTAACGACGAAACTGGCTAAAATAAGTAAACAGGTAACGTCTATTGAATTAGACAGTCATCTATTCAACTTATCGTCAGAAAAAT 1185
L. garvieae     ATACCGTTTACGAAATTGGAACAGGTAAAGGGCATTTAACGACGAAACTGGCTAAAATAAGTAAACAGGTAACGTCTATTGAATTAGACAGTCATCTATTCAACTTATCGTCAGAAAAAT 1187
P. acidilactici ATACCGTTTACGAAATTGGAACAGGTAAAGGGCATTTAACGACGAAACTGGCTAAAATAAGTAAACAGGTAACGTCTATTGAATTAGACAGTCATCTATTCAACTTATCGTCAGAAAAAT 1173
S. parauberis   ATACCGTTTACGAAATTGGAACAGGTAAAGGGCATTTAACGACGAAACTGGCTAAAATAAGTAAACAGGTAACGTCTATTGAATTAGACAGTCATCTATTCAACTTATCGTCAGAAAAAT 1081
                ************************************************************************   *********************************************

L. lactis       TAAAACTGAATACTCGTGTCACTTTAATTCACCAAGATATTCTACAGTTTCAATTCCCTAACAAACAGAGGTATAAAATTGTTGGGAATATTCCTTACCATTTAAGCACACAAATTATTA 1302
E. faecalis     TAAAACTGAATACTCGTGTCACTTTAATTCACCAAGATATTCTACAGTTTCAATTCCCTAACAAACAGAGGTATAAAATTGTTGGGAATATTCCTTACCATTTAAGCACACAAATTATTA 1305
L. garvieae     TAAAACTGAATACTCGTGTCACTTTAATTCACCAAGATATTCTACAGTTTCAATTCCCTAACAAACAGAGGTATAAAATTGTTGGGAGTATTCCTTACCATTTAAGCACACAAATTATTA 1307
P. acidilactici TAAAACTGAATACTCGTGTCACTTTAATTCACCAAGATATTCTACAGTTTCAATTCCCTAACAAACAGAGGTATAAAATTGTTGGGAATATTCCTTACCATTTAAGCACACAAATTATTA 1293
S. parauberis   TAAAACTGAATACTCGTGTCACTTTAATTCACCAAGATATTCTACAGTTTCAATTCCCTAACAAACAGAGGTATAAAATTGTTGGGAGTATTCCTTACCATTTAAGCACACAAATTATTA 1201
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L. lactis       AAAAAGTGGTTTTTGAAAGCCGTGCGTCTGACATCTATCTGATTGTTGAAGAAGGATTCTACAAGCGTACCTTGGATATTCACCGAACACTAGGGTTGCTCTTGCACACTCAAGTCTCGA 1422
E. faecalis     AAAAAGTGGTTTTTGAAAGCCGTGCGTCTGACATCTATCTGATTGTTGAAGAAGGATTCTACAAGCGTACCTTGGATATTCACCGAACACTAGGGTTGCTCTTGCACACTCAAGTCTCGA 1425
L. garvieae     AAAAAGTGGTTTTTGAAAGCCATGCGTCTGACATCTATCTGATTGTTGAAGAAGGATTCTACAAGCGTACCTTGGATATTCACCGAACACTAGGGTTGCTCTTGCACACTCAAGTCTCGA 1427
P. acidilactici AAAAAGTGGTTTTTGAAAGCCGTGCGTCTGACATCTATCTGATTGTTGAAGAAGGATTCTACAAGCGTACCTTGGATATTCACCGAACACTAGGGTTGCTCTTGCACACTCAAGTCTCGA 1413
S. parauberis   AAAAAGTGGTTTTTGAAAGCCATGCGTCTGACATCTATCTGATTGTTGAAGAAGGATTCTACAAGCGTACCTTGGATATTCACCGAACACTAGGGTTGCTCTTGCACACTCAAGTCTCGA 1321
                ********************* **************************************************************************************************

L. lactis       TTCAGCAATTGCTTAAGCTGCCAGCGGAATGCTTTCATCCTAAACCAAAAGTAAACAGTGTCTTAATAAAACTTACCCGCCATACCACAGATGTTCCAGATAAATATTGGAAGCTATATA 1542
E. faecalis     TTCAGCAATTGCTTAAGCTGCCAGCGGAATGCTTTCATCCTAAACCAAAAGTAAACAGTGTCTTAATAAAACTTACCCGCCATACCACAGATGTTCCAGATAAATATTGGAAGCTATATA 1545
L. garvieae     TTCAGCAATTGCTTAAGCTGCCAGCGGAATGCTTTCATCCTAAACCAAAAGTAAACAGTGTCTTAATAAAACTTACCCGCCATACCACAGATGTTCCAGATAAATATTGGAAGCTATATA 1547
P. acidilactici TTAAGCAATTGCTTAAGCTGCCAGCTGAATGCTTTCATCCTAAACCAAAAGTAAACAGTGCCTTAATAAAACTTACCCGCCATACCACAGATGTTCCAGATAAATATTGGAAACTATATA 1533
S. parauberis   TTCAGCAATTGCTTAAGCTGCCAGCGGAATGCTTTCATCCTAAACCAAAAGTAAACAGTGTCTTAATAAAACTTACCCGCCATACCACAGATGTTCCAGATAAATATTGGAAGCTATATA 1441
                ** ********************** ********************************** *************************************************** *******

L. lactis       CGTACTTTGTTTCAAAATGGGTCAATCGAGAATATCGTCAACTGTTTACTAAAAATCAGTTTCATCAAGCAATGAAACACGCCAAAGTAAACAATTTAAGTACCATTACTTATGAGCAAG 1662
E. faecalis     CGTACTTTGTTTCAAAATGGGTCAATCGAGAATATCGTCAACTGTTTACTAAAAATCAGTTTCATCAAGCAATGAAACACGCCAAAGTAAACAATTTAAGTACCATTACTTATGAGCAAG 1665
L. garvieae     CGTACTTTGTTTCAAAATGGGTCAATCGAGAATATCGTCAACTGTTTACTAAAAATCAGTTTCATCAAGCAATGAAACACGCCAAAGTAAACAATTTAAGTACCGTTACTTATGAGCAAG 1667
P. acidilactici CGTACTTTGTTTCAAAATGGGTCAATCGAGAATATCGTCAACTGTTTACTAAAAATCAGTTTCATCAAGCAATGAAATACGCCAAAGTAAACGATTTAAGTACCGTTACTTATGAGCAAG 1653
S. parauberis   CGTACTTTGTTTCAAAATGGGTCAATCGAGAATATCGTCAACTGTTTACTAAAAATCAGTTTCATCAAGCAATGAAACACGCCAAAGTAAACAATTTGAGTACCGTTACTTATGAGCAAG 1561
                ***************************************************************************** ************** **** ****** ***************
                                             ORF2 erm(B) stop┐    ┌ ORF3 start
L. lactis       TATTGTCTATTTTTAATAGTTATCTATTATTTAACGGGAGGAAATAATTCTATGAGTCGCTTTTTTAAATTTGGAAAGTTACACGTTACTAAAGGGAATGGAGATAAATTATTAGATATA 1782
E. faecalis     TATTGTCTATTTTTAATAGTTATCTATTATTTAACGGGAGGAAATAATTCTATGAGTCGCTTTTTTAAATTTGGAAAGTTACACGTTACTAAAGGGAATGGAGATAAATTATTAGATATA 1785
L. garvieae     TATTGTCTATTTTTAATAGTTATCTATTATTTAACGGGAGGAAATAATTCTATGAGTCGCTTTTTTAAATTTGGAAAGTTACACGTTACTAAAGGGAATGGAGATAAATTATTAGATATA 1787
P. acidilactici TATTGTCTATTTTTAATAGTTATCTATTATTTAACGGGAGGAAATAATTCTATGAGTCGCTTTTTTAAATTTGGAAAGTTACACGTTACTAAAGGGAATGGAGATAAATTATTAGATATA 1773
S. parauberis   TATTGTCTATTTTTAATAGTTATCTATTATTTAACGGGAGGAAATAATTCTATGAGTCGCTTTTGTAAATTTGGAAAGTTACACGTTACTAAAGGGAATGTAGATAAATTATTAGGTATA 1681
                ***************************************************************** ********************************** ************** ****
                              ORF3 stop┐                                                                         ┌331bp repeat start
L. lactis       CTACTGACAGCTTCCAAGAAGCTAAAGAGGTCCCTAGCGCCTACGGGGAATTTGTATCGATAAGGGGTACAAATTCCCACTAAGCGCTCGGGACCCCTTGTAGGAAAATGTCCTAAGTGT 1902
E. faecalis     CTACTGACAGCTTCCAAGAAGCTAAAGAGGTCCCTAGCGCCTACGGGGAATTTGTATCGATAAGGGGTACAAATTCCCACTAAGCGCTCGGGACCCCTTGTAGGAAAATGTCCTAAGTGT 1905
L. garvieae     CTACTGACAGCTTCCAAGAAGCTAAAGAGGTCCCTAGCGCCTACGGGGAATTTGTATCGATAAGGGGTACAAATTCCCACTAAGCGCTCGGGACCCCTTGTAGGAAAATGTCCTAAGTGT 1907
P. acidilactici CTACTGACAGCTTCCAAGAAGCTAAAGAGGTCCCTAGCGCCTACGGGGAATTTGTATCGATAAGGGGTACAAATTCCCACTAAGCGCTCGGGACCCCTTGTAGGAAAATGTCCTAAGTGT 1893
S. parauberis   CTACTGACAGCTTCCAAGGAGCTAAAGAGGTCCCTAGCGCCTACGGGGAATTTGTATCGATAAGGGGTACAAATTCCCACTAAGCGCTCGGGACCCCTTGTAGGAAAATGTCCTAAGTGT 1801
                ****************** *****************************************************************************************************

L. lactis       GGCAACAATATTGTATTAAAAAAATCGTTTTATGGTTGTTCAAATTATCCTGAATGTAAGTTTACTTTAGCTGAACATTTTAGAAAGAAAAAACTAACCAAAACGAATGTAAAAGAATTA 2022
E. faecalis     GGCAACAATATTGTATTAAAAAAATCGTTTTATGGTTGTTCAAATTATCCTGAATGTAAGTTTACTTTAGCTGAACATTTTAGAAAGAAAAAACTAACCAAAACGAATGTAAAAGAATTA 2025
L. garvieae     GGCAACAATATTGTATTAAAAAAATCGTTTTATGGTTGTTCAAATTATCCTGAATGTAAGTTTACTTTAGCTGAACATTTTAGAAAGAAAAAACTAACCAAAACGAATGTAAAAGAATTA 2027
P. acidilactici GGCAACAATATTGTATTAAAAAAATCGTTTTATGGTTGTTCAAATTATCCTGAATGTAAGTTTACTTTAGCTGAACATTTTAGAAAGAAAAAACTAACCAAAACGAATGTAAAAGAATTA 2013
S. parauberis   GGCAACAATATTGTATTAAAAAAATCGTTTTATGGTTGTTCAAATTATCCTGAATGTACGTTCACTTTAGCTGAACATTTTAGAAAGAAAAAACTCACCAAAACAAATGTAAAAGAATTA 1921
                ********************************************************** *** ******************************** ******** ***************

L. lactis       CTGGAGGGAAAAGAAACCCTGGTAAAAGGAATCAAAAACAAAGAGAAAAAGCCCTACAATGCCGTTGTAAAAATTGGGGAAAAGGGATATATTGATTTTATCTCTTTTTCAAAATAAACA 2142
E. faecalis     CTGGAGGGAAAAGAAACCCTGGTAAAAGGAATCAAAAACAAAGAGAAAAAGCCCTACAATGCCGTTGTAAAAATTGGGGAAAAGGGATATATTGATTTTATCTCTTTTTCAAAATAAACA 2145
L. garvieae     CTGGAGGGAAAAGAAACCCTGGTAAAAGGAATCAAAAACAAAGAGAAAAAGCCCTACAATGCCGTTGTAAAAATTGGGGAAAAGGGATATATTGATTTTATCTCTTTTTCAAAATAAACA 2147
P. acidilactici CTGGAGGGAAAAGAAACCCTGGTAAAAGGAATCAAAAACAAAGAGAAAAAGCCCTACAATGCCGTTGTAAAAATTGGGGAAAAGGGATATATTGATTTTATCTCTTTTTCAAAATAAACA 2133
S. parauberis   CTAGAGGGAAAAGAAACCCTGGTAAAAGGAATCAAAACGAAAGATAGAAAGTCCTACAATGCCGTTGTAAAAATCGGAGAAAAGGGATATATTGATTTTATCTCTTTTTCAAAATAAGCA 2041
                ** **********************************  ***** * **** ********************** ** *************************************** **
                                                 331bp repeat stop┐                                 ┌ORF4 start
L. lactis       TAAAAGCCCTTTAAAGAGGGCTTTTATATATTAATCACAAATCACTTATC---------------------ACAAATCACTTGTGATTTGTGATTCTTAATGATACAATATTACTATACA 2241
E. faecalis     TAAAAGCCCTTTAAAGAGGGCTTTTATATATTAATCACAAATCACTTATCACAAATCACAAGTGATTAATCACAAATCACTTGTGATTTGTGATTCTTAATGATACAATATTACTATACA 2265
L. garvieae     TAAAAGCCCTTTAAAGAGGGCTTTTATATATTAATCACAAATCACTTATCACAAATCACAAGTGATTAATCACAAATCACTTGTGATTTGTGATTCTTAATGATACAATATTACTATACA 2267
P. acidilactici TAAAAGCCCTTTAAAGAGGGCTTTTATATATTAATCACAAATCACTTATCACAAATCACAAGTGATTAATCACAAATCACTTGTGATTTGTGATTCTTAATGATACAATATTACTATACA 2253
S. parauberis   TAAAAGCCCTTTAAAGAGGGCTTTTATATATTAATCACAAATCACTTATCACAAATCACAAGTGATTAATCACAAATCACTTGTGATTTGTGATTCCTAATGATACAATATTACTATACA 2161
                **************************************************                     ************************* ***********************

L. lactis       AAAAAAGAATGGGGCGTAGTTATGGAGAAGGAAAAACTAAAAATACTTGAAGAATTAAGACGTATTTTAAACAATAAAAATGAAGCAATTATTATCTTGAATAATTACTTTAAAGGTGGT 2361
E. faecalis     AAAAAAGAATGGGGCGTAGTTATGGAGAAGGAAAAACTAAAAATACTTGAAGAATTAAGACGTATTTTAAACAATAAAAATGAAGCAATTATTATCTTGAATAATTACTTTAAAGGTGGT 2385
L. garvieae     AAAAAAGAATGGGGCGTAGTTATGGAGAAGGAAAAACTAAAAATACTTGAAGAATTAAGACGTATTTTAAACAATAAAAATGAAGCAATTATTATCTTGAATAATTACTTTAAAGGTGGT 2387
P. acidilactici AAAAAAGAATGGGGCGTAGTTATGGAGAAGGAAGAACTCAAAATACTTGAAGAATTAAGACGTATTTTAAACAGTAAAAATGAAGCAATTGTTATCTTAAACAATTATTTTAAAGGTGGT 2373
S. parauberis   AAAAAAGAATGGGGCGTAGTTATGGAGAAGGAAGAACTCAAAATACTTGAAGAATTAAGACGTATTTTAAACAGTAAAAATGAAGCGATTGTTATCTTAAACAATTATTTTAAAGGTGGT 2281
                ********************************* **** ********************************** ************ *** ******* ** ***** ************

L. lactis       GTTGGAAAGTCCAAATTATCGACTATGTTTGCTTACTTGACAGACAAATTGAATTTAAAAGTTTTAATGATCGATAAGGACTTACAGGCAACATTGACAAAAGACTTAGCAAAAACTTTT 2481
E. faecalis     GTTGGAAAGTCCAAATTATCGACTATGTTTGCTTACTTGACAGACAAATTGAATTTAAAAGTTTTAATGATCGATAAGGACTTACAGGCAACATTGACAAAAGACTTAGCAAAAACTTTT 2505
L. garvieae     GTTGGAAAGTCCAAATTATCGACTATGTTTGCTTACTTGACAGACAAATTGAATTTAAAAGTTTTAATGATCGATAAGGACTTACAGGCAACATTGACAAAAGACTTAGCAAAAACTTTT 2507
P. acidilactici GTTGGAAAGTCAAAATTATCTACTATGTTTGCTTATTTGACAGACAAATTTAATTTAAAAGTTTTAATGATCGATAAGGACTTACAAGCAACATTGACAAAAGACTTAGCAAAAACATTT 2493
S. parauberis   GTTGGAAAGTCAAAATTATCGACTATGTTTGCTTACTTGACAGACAAATTTAATTTAAAAGTTTTAATGATCGATAAGGACTTACAAGCAACATTGACAAAAGACTTAGCAAAAACATTT 2401
                *********** ******** *************** ************* *********************************** ***************************** ***

L. lactis       GAGGTAGAATTGCCACGTGTCAATTTTTATGAAGGCTTGAAAAATGGAAACTTGGCTTCTTCTATTGTTCATTTGACTGATAATTTAGACTTGATCCCTGGCACGTTTGATTTGATGTTA 2601
E. faecalis     GAGGTAGAATTGCCACGTGTCAATTTTTATGAAGGCTTGAAAAATGGAAACTTGGCTTCTTCTATTGTTCATTTGACTGATAATTTAGACTTGATCCCTGGCACGTTTGATTTGATGTTA 2625
L. garvieae     GAGGTAGAATTGCCACGTGTCAATTTTTATGAAGGCTTGAAAAATGGAAACTTGGCTTCTTCTATTGTTCATTTGACTGATAATTTAGACTTGATCCCTGGCACGTTTGATTTGATGTTA 2627
P. acidilactici AAGGTAGAATTGCCACGTGTCAATTTTTATGAAGGCTTGAAAAATGGAAACTTGGCTTCTTCTATTATTCATTTGACTGATAATTTAGACTTGATCCCTGGCACGTTTGATTTGATGTTA 2613
S. parauberis   AAGGTAGAATTGCCACGTGTTAATTTTTATGAAGGACTGAAAAATGGAAACTTGGCTTCTTCTATTGTTCATTTGACTGATAATTTAGACTTGATCCCTGGCACGTTTGATTTGATGTTA 2521
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L. lactis        CTGCCAAAATTAACTCGCTCATGGACTTTTGAAAATGAAAGTAGATTGCTTGCTACTCTTTTAGCACCTTTAAAAAGTGACTATGATCTCATTATTATTGATACTGTACCAACGCCAAGC 2721
E. faecalis      CTGCCAAAATTAACTCGCTCATGGACTTTTGAAAATGAAAGTAGATTGCTTGCTACTCTTTTAGCACCTTTAAAAAGTGACTATGATCTCATTATTATTGATACTGTACCAACGCCAAGC 2745
L. garvieae      CTGCCAAAATTAACTCGCTCATGGACTTTTGAAAATGAAAGTAGATTGCTTGCTACTCTTTTAGCACCTTTAAAAAGTGACTATGATCTCATTATTATTGATACTGTACCAACGCCAAGC 2747
P. acidilactici  TTACCAAAATTAACTCGCTCATGGACTTTTGAAAATGAAAGTAGATTGCTTGCTACTCTTTTAGCACCTTTAAAAAGTGACTATGATCTCATTATTATTGATACTGTACCAACGCCAAGC 2733
S. parauberis    CTGCCAAAATTAACTCGCTCATGGACTTTTGAAAATGAAAGTAGATTGCTTGCTACTCTTTTAGCACCTTTAAAAAGTGACTATGATCTCATTATTATGGATACTGTACCACCGCCAAGC 2641
                 * ************************************************************************************************ ************ ********

L. lactis        GTTTATACAAATAATGCAATCGTGGCGAGTGATTAC- 2757
E. faecalis      GTTTATACAAATAATGCAATCGTGGCGAGTGATTACG 2782
L. garvieae      GTTTATACAAATAATGCAATCGTGGCGAGTGATTAC- 2783
P. acidilactici  GTTTATACAAATAATGCAATCGTGGCGAGTGATTAC- 2769
S. parauberis    GTTTATCCAAATAATGCAATCGTGGC-AGTGAATACC 2677
                 ****** ******************* ***** ***
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B

Bacterial species	%identity	%similarity (vs. *S. parauberis*)
P. acidilactici	93.3	93.4
E. faecalis	93.0	93.1
L. garvieae	92.9	93.0
L. lactis	92.0	92.0

C

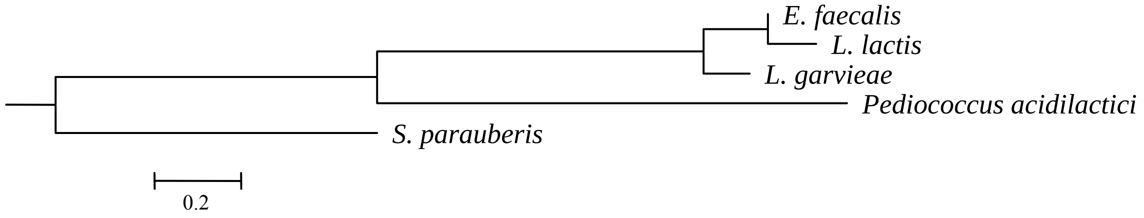

Fig. 3-4. (A) Alignment of the nucleotide sequences of the 2677bp *erm*(B) gene region from *S. parauberis* NUF927 with those of *Lactococcus lactis* plasmid pI1o8 (accession number AJ549242), *E. faecalis* plasmid pRE25 (accession number X92945), *L. garvieae* plasmid pKL0018 (accession number AB290882), and *Pediococcus acidilactici* plasmid pEOC01 (accession number DQ220741) generated using the ClustalW software. The asterisk (*) shows sequences that are the same as those of the *erm*(B) gene regions. The organism is given on the left of each sequence lane. The sequence number is given on the right of each sequence lane. (B) Degree of homology of the *erm*(B) gene region in *S. parauberis* NUF927 to the related region in other bactria. (C) Phylogenetic trees of the *erm*(B) gene region drawn by the maximum likelihood method using MEGA (version 4.0) software.

Chapter 4

Characterization of resistance determinants in *Streptococcus parauberis* setorype II strains

In chapter 1, all of the serotype II strains were EM (MICs=0.25-1 μg/mL) and OTC (MICs=32μg/mL) intermediately resistant. Furthermore, in chapter 2, although positive amplifications were obtained with Tn*916*-family specific primers for *tet*(M) (tetracycline resistance), *xis* (excisionase)and *int* (integrase) in these strains, the EM resistance genes could not be detected. It means these strains may have different mechanisms for EM resistance. According to the numerous documents, Tn*916* family have acquired additional resistance determinants, such as Tn*1545* [EM and KM resistances], Tn*6002* [EM resistance] and Tn*6003* [EM, KM and aminoglucosides resistances] (Caillaud *et al.*, 1987; Warburton *et al.*, 2007; Cochetti *et al.*, 2008). A considerable relationship between Tn*916*-like element and EM resistance gene might exist in serotype II because no plasmid was detected. In another word, the undetected EM resistance determinant coexiting with *Tet*(M) might be mediated by the Tn*916*-like element.

Based on this result, primers according to the sequences of Tn*916* (accession no. U09422) in databases were designed to amplify the full length of Tn*916*-like element. The aim of this charpter is to characterize the Tn*916*-like element of *S. parauberis* by comparing the total DNA sequence with that of Tn*916* from *E. faecalis* and the insertion site of the Tn*916*-like elements in the chromosomes of serotype II strains. The relationship between serotype I and II strains concerning the insertion site was also characterized.

Materials and Methods

Strains, medium and DNA

Thirty-two *S. parauberis* serotype II strains used in this study were isolated at flounder aquaculture sites located in Kagawa (8 strains), Ehime (9), Kumamoto (1), Oita (10), Kagoshima (1) and Nagasaki (3) Prefectures in 2002 to 2008. *S. parauberis* NUF1003 and NUF1049 were used as the representative strains of serotype I and II, respectively. *E. faecalis* CG110 which harbored Tn*916* was used as the reference strain (Shimoji *et al*., 1994) and *E. faecalis* FA2-2 as the recipient strain in conjugal transfer experiments (Shiojima *et al*., 1997). These strains were grown in Todd-Hewitt broth (THB; Difco Laboratories). Chromosomal and plasmid DNA of each strain were prepared using Wizard Genomic DNA Purification kit (Promega) and QIAprep miniprep kit (QIAGEN), respectively.

PCR and inverse PCR amplification

The primer pairs used in this study are listed in Table 4-1.*TaKaRa Ex Taq* Hot start version (TaKaRa Bio) was used for PCR, except for Tn*916*, for which *TaKaRa LA* (Long and accurate) *Taq* Hot start version was used. The Tn-upstream and Tn-downstream regions were amplified by inverse PCR (Ochman *et al*. 1988). In order to reduce the contamination in products, nested PCR was carried out to amplify secondary targets within the inverse PCR products. The loci of primers are shown in Fig. 4-1. The PCR was performed on C1000 Thermal Cycler (Bio-Rad).

Southern blot hybridization

Southern blot hybridization analysis was carried out with DIG-labeled probes [*tet*(M)-*int* and Tn*916*-like element] (Table 4-1) and detection system (Roche). Chromosomal DNA (1.5 μg) extracted from serotype II strains of *S. parauberis* and *E.*

faecalis CG110 were digested to completion at 37℃ with *Hinc*II, *Hind*III or *Sau*3AI (TaKaRa Bio). The digested DNA were electrophoresed on 0.7% agarose gel and stained with ethidium bromide. After depurination, denaturation and neutralization of the gel, DNA were transferred to Hybond N^+ membrane (GE Healthcare) by capillary blotting overnight. Hybridization and washings were under stringent conditions, and chemiluminescent reaction was carried out with AP-labeled anti-DIG antibody and CDP-Star (Roche).

Sequencing analysis of Tn*916*-like element and flanking region

PCR products (Tn A, B, C and D) and inverse PCR products (Tn-upstream 2 and Tn-downstream 2) from *S. parauberis* NUF1049 and the relative flanking region of Tn*916*-like element from *S. parauberis* NUF1003 were cloned into pGEM-T Easy vector (Promega) and transformed into *E.coli* JM109 Competent Cells (Promega). DNA sequencing was carried out with Big Dye Terminator v3.1 Cycle Sequencing kit and ABI Prism 3100 Genetic Analyzer (Applied Biosystems). Sequences were assembled and analyzed using DNASIS program (Hitachi Software Engineering) and BLAST at the National Center for Biotechnology Information (NCBI) website (www.ncbi.nlm.nih.gov./BLAST). Alignment program ClustalW2 was used for DNA and protein multiple sequence alignment (Larkin *et al.*, 2007).

Conjugative transfer experiments

Filter mating assays was performed by using logarithmic-phase broth cultures of six *S. parauberis* serotype II strains (one strain per prefecture) as donors and *E. faecalis* FA2-2 as recipient. Mixture of donor and recipient cells at a ratio of one donor (50 *μ*L) per 10 recipients (500 *μ*L) was collected on a sterilized membrane filter (0.45 *μ*m) (MILLIPORE), and the filter was incubated on a 5% horse blood agar plate at 28℃ overnight. Transconjugants were selected on TH agar plates containing oxytetracycline (8 *μ*g/mL), rifampicin (25 *μ*g/mL) and fusidic acid (25 *μ*g/mL).

Nucleotide sequence accession number

The nucleotide sequences of Tn*916*-like element and the flanking region of the strain NUF1049 are available from the DDBJ, EMBL and GenBank nucleotide database under the accession number AB468159.

Results

Screening for Tn*916*-like element among *S. parauberis* serotype II strains

The 32 serotype II strains obtained single PCR products [*tet*(M)-*int*] of around 4.5 kbp with the primer pair tet(M)-FW—Int-RV (Table 4-1). In Southern blot hybridization analysis, the DIG-labelled *tet*(M)-*int* was found to uniformly hybridize to 7.2 kbp *Hin*dIII-digested fragments of the chromosomal DNA of the serotype II strains (Fig. 4-2). The results suggested that resistance to tetracycline in *S. parauberis* serotype II strains is due to the presence of one copy of a chromosomally borne element structurally related to Tn*916*.

Analysis of the nucleotide sequences of the *tet*(M)-*int* from two strains chosen at random showed that their sequences were identical. Comparison of the sequences between *tet*(M)-*int* and the corresponding region of Tn*916* of *E. faecalis* DS16 (Flannagan *et al*., 1994; accession number U09422) also showed that they were identical.

Based on this similarity, primer pair of Tn916-FW and Tn916-RV (Table 4-1) was designed from the database to amplify the full length of Tn*916*-like element. Positive amplifications of the whole length were obtained when chromosomal DNA from serotype II strains were used as template for LA PCR. The size of amplifications was the same as those from *E. faecalis* CG110, the positive control of Tn*916* (data not shown). These evidences indicate that the resistance elements harbored by serotype II strains are closely related to Tn*916*.

Nucleotide Sequence analysis of Tn*916*-like element

Positive amplifications were also detected when four primer pairs [Table 4-1, TnA-D] based on the four overlapped fragments of Tn*916* were used. Locations of PCR amplifications are shown in Fig. 4-1 (A). Tn*916*-like element harbored in serotype II strains were constructed by these four fragments which could span the Tn*916*-like element. PCR products of these four fragments were cloned into pGEM-T Easy vector (Promega) and sequenced. The size of entire Tn*916*-like element was found to be 18,031 bp in length. Analysis and comparison of the sequencing result showed that the element was 99.8% identical to that of Tn*916* original from *E. feacalis* DS16. Tn*916*-like element encodes 22 ORFs as Tn*916*.

Multiple DNA alignment of Tn*916* sequences

The nucleotide sequences of *S. parauberis* NUF1049 Tn*916*-like element were aligned with those of *S. agalactiae* 2603V/R Tn*916*-like element, *E. faecalis* plasmid pCF10 Tn*925*, E. *faecalis* DS16 Tn*916*, and *S. suis* Tn*916*, using the ClustalW2 software (Fig. 4-3). All the sequences possess the genetic characterizations for Tn*916*: *tet*(M), *int* and *xis*. However, *S. parauberis* Tn*916* is more similar to Tn*916*-like element of *S. agalactiae* than to *E. faecalis* DS16 Tn*916*, *E. faecalis* plasmid pCF10 Tn*925* and *S. suis* Tn*916*.

Structural comparison of the Tn*916*-like element harbored by the *S. parauberis* serotype II strains

All of the serotype II strains showed the same *Sau*3AI and *Hin*dIII restriction patterns as Tn*916* by Southern blot hybridization analysis. This strongly suggested that all of the serotype II strains harbored complete copies of Tn*916*-like element and the element existed in the same chromosomal location of all of serotype II strains (Fig. 4-4). However, *Hin*cII hybridizing patterns of serotype II strains were diverse, namely two patterns existed among serotype II strains (Fig. 4-5). Only one strain derived from

Kagoshima Prefecture out of 32 serotype II strains showed pattern I which consisted of 5.6-, 4.9-, 3.0-, 1.7-,1.2-, 0.9- and 0.4-kbp *Hinc*II fragments like Tn*916*, whereas the rest strains showed pattern II that contained a 10.5-kbp fragment instead of 5.6 kbp and 4.9 kbp fragments. In these two patterns, *tet*(M) gene located in 10.5 and 4.9 kbp *Hinc*II fragments, respectively.

Characterization of the flanking regions and termini of the Tn*916*-like element

Restriction analysis of the Tn*916*-like element constructs with *Hin*dIII had shown that the Tn*916*-like element insertions were in the same orientation since 15.2 and 7.2kbp fragments had been obtained uniformly among all of serotype II strains. This further proved that a single *Hin*dIII restriction site exist in the element. Cloning of left and right flanking fragments adjacent to Tn*916*-like element was accomplished through circularization of those two *Hin*dIII restriction digests [Fig. 4-1 (B)]. Inverse and nested PCR amplifications were carried out by using primer pairs listed in Table 4-1. Inverse and nested PCR of the left flanking region were expected to provide 4.7 and 4.5kbp fragments, respectively. Furthermore, 4.5kbp and 2.7kbp fragments of the related right flanking region were also obtained. Sequence analysis of the flanking regions of the Tn*916*-like element showed that insertion site of Tn*916*-like element of the host chromosome is the A-T rich regions. GenBank ORF finder (http://www.ncbi.nlm.nih.gov/gorf/gorf.html) and homology search revealed that the amino acid sequences coded by putative CDSs (coding sequences) of ORF Sp-O1, -O2 and -O3 have 68%, 72% and 84% identity to transcriptional regulator, dihydroxyacetone kinase (accession number CP000262) and threonyl-tRNA synthetase (accession number AE014074) from *S. pyogenes*, respectively [Fig.4-6(A)]. It is interesting that the insertion of the Tn*916*-like element did not interrupt any ORF of chromosomal DNA of *S. parauberis* serotype II strains.

Based on the above results, the supposition that the Tn*916*-like element inserted in the similar sites among serotype II strains was testified. Two primer pairs were chosen, one is the left terminus primer pair, Tn-5R located on the left internal position of

Tn*916*-like element and Tn-11F-4F located on the upstream of Tn*916*-like element, and the other is the right terminus primer pair, Tn-2F located on the right internal position of Tn*916*-like element and Tn-3R-2R located on the downstream of Tn*916*-like element [Table 4-1; Fig. 4-1 (A)]. The PCR amplification results proved that the Tn*916*-like element located in the uniform location in all of serotype II strains tested.

Since Tn*916*-like element exists in all of chromosomal DNA of serotype II strains at the similar locations, it is necessary to clarify the relationship between serotype I and II strains concerning the insertion sites of Tn*916*-like elements. The primers Tn-11F-4F located on the left side and Tn-3R-2R located on the right side of the flanking region were selected for amplify the corresponding region of serotype I strains [Table 4-1, Fig. 4-6 (A)]. Sequencing analysis of the related flanking region from a serotype I strain showed that the insertion site was generated 6 bp [ATCATA, underlined in Fig. 4-6 (B)] and adjacent to the left end of Tn*916*-like element in the integrated state in serotype II strain. The 6bp sequence [TATATA, emphasized by the dot line box in Fig. 4-6 (B)] represented the initial target sequence for the insertion of Tn*916*-like element.

Conjugative transfer of Tn*916*-like element

Six strains isolated from different prefectures were used as donor in filter mating experiments. No detectable transfer of Tn*916*-like element from any donor was obtained by using *E. faecalis* FA2-2 as the recipient.

Discussion

This study elucidates the complete organization and structure of the Tn*916*-like element observed in *S. parauberis* serotype II strains examined. Therefore, our data provide evidence that Tn*916*-like element with *tet*(M) gene plays as an important selective factor that provides considerable advantages for the emergence and spread of *S. parauberis* serotype II. The widespread use of tetracycline in cultured Japanese flounder could provide the selective pressure for clone amplification and spreading, thus contributing to the outbreak of serotype II through Tn*916*-like element.

Tn*916*-like element is homology with Tn*916* but differing from it in structure detected in almost all the strains (31/32) studied here. It is worthy to note that the sizes of internal *Hinc*II fragments of the Tn*916* restriction map are 5.6, 4.9, 1.7, 1.2 and 0.4 kbp, respectively (Senghas *et al.*, 1988). *tet*(M) gene is located on 4.9 kbp fragment which is present in Tn*916* and in most Tn*916*-like structures (Bentorcha *et al.*, 1992). However, this Tn*916* structural character could be observes only in one strain NUF1048 isolated from Kagoshima Prefecture (Fig. 4-5-Lane 1). The difference is due to a nucleotide change of the restriction site located in ORF14 from GTCAAC to GTCACC for the 31 strains. It is interesting that the similar structure was also detected on the chromosomes or plasmids in enterococcal strains (Bentorcha *et al.*, 1992). These results suggested that the diverse structures of Tn*916*-like element exist among serotype II isolates.

int and *xis* genes are involved in the conjugative transposition mechanism in Tn*916* family (Rice, 1998). In this study, our PCR results, sequencing data and Southern hybridization results of the Tn*916*-like element have revealed the presence of these two genes in all of serotype II strains. Based on these results, the element would be predicted to be conjugal transfer. However, horizontal transfer of Tn*916*-like element was not detected from serotype II strains to recipient by filter mating assays. Tn*916*-family conjugative transposons have been found in a broad variety of gram-positive and gram-negative organisms and become important vehicles for disseminating antimicrobial resistance through cell to cell contact (Rice, 1998). Conjugative transposition of Tn*916* is via an excision-insertion mechanism with three stages: excision and circularization, conjugal transfer and integration (Caparon and Scott, 1989). Excision and insertion of conjugative transposons began with generating staggered nicks of 6 bp (Scott, 1992). The ligated staggered nicks yield heteroduplex in both circular intermediate and target site. The coupling sequence is the 6bp junction sequences comprising heteroduplex which will be resolved by replication. The nucleotide content of the coupling sequences (6bp) flanking the inserted transposon plays a frequency-determining role (Jaworski and Clewell, 1994). Therefore, transposition from different donors into the same target site occurs at widely differing frequencies ranging from less than 10^{-8} to greater than 10^{-4} per donor. According to this mechanism, our sequence data of serotype I and II provided evidence that the

Tn*916*-like element introduced a coupling sequence (ATCATA) derived from its previous host target sequence. Aanalyzing the coupling sequence adjacent to Tn*916*-like element (ATCATA, adjacent to the left end of element; TATATA, adjacent to the right end of element) in a serotype II strain demonstrated that the coupling sequences might result in the extreme low transfer frequency in the second-generation transposition events.

In conclusion, this chapter reported that all of *S. parauberis* serotype II strains from Japanese flounder harbored Tn*916*-like element. These transposons expressed tetracycline resistance conferred by *tet*(M) gene and inserted into an A-T rich region of chromosomal DNA of serotype II strains. With the complete DNA-sequencing of the Tn*916*-like element and flanking regions, studies of spreading mechanism of this element are possible to further elucidate the molecular interaction between Tn*916*-like element and the host. Furthermore, future studies on the presence of Tn*916*-like element or its variants in a broader range of strains of *S. parauberis* should be enhanced, since it is important to understand the prevalence and transposon-mediated resistance to tetracycline for the reason of clinical importance.

Conclusion

1. All of serotype II strains possessed the Tn*916*-like element harboring *tet*(M) gene.
2. The *S. parauberis* Tn*916* shares 99% homology with that from *E. faecalis* DS16 strain (accession number U09422).
3. Insertion site of Tn*916*-like element was the region with high A-T content and generated 6 bp (ATCATA) attaching at the upstream end of Tn*916*-like element.

Table 4-1. Oligonucleotides used for PCR

Amplicon	Primer designation	Primer sequence (5′ to 3′)	Source	Annealing temperature (℃)
tet(M)-*int* *	*tet*(M)-FW Int-RV	GTTAAATAGTGTTCTTGGAG CCATAGGAACTTGACGTTCG	GenBank (U90422)	57
Tn*916* *	Tn916-FW Tn916-RV	CTGGCGAGGATAAAGTCGTTCAGCG CAAACTATGTGGAGTGATGTGTGGC	GenBank (U90422)	68
Tn A	Tn916-FW Tn2-AR	CTGGCGAGGATAAAGTCGTTCAGCG CGTGCCACGTCATACATATCATCAC	GenBank (U90422)	56
Tn B	TnB-1F Tn2-BR	CTTCCATTGGCGAACTCAATC GAGTGATCTGCTTGTGTCCTTGCAG	GenBank (U90422)	61
Tn C	TnC-1F TnC-1R	CGATTCTCAAAGTGTGGGAAG CCAAGAACACTATTTAACTTC	GenBank (U90422)	61
Tn D	*tet*(M)-FW TnE-1R	GTTAAATAGTGTTCTTGGAG GTCATGGCTATATTAGCATGTC	GenBank (U90422)	65
Tn-upstream 1†	Tn-10F Tn-12R	CTATCCTACAGCGACAGCCAGTGAACTTTC GCTGGCAGGAATACTTACTTGAATCATGCG	this study	50
Tn-upstream 2‡	Tn-11F Tn-5R	GTATCGCTGACAGTGGAGTATATCGACCAG TTCTTCGCTGAACGACTTTATCCTCGCCAG	this study	63
Tn-downstream 1†	Tn-1F Tn-4R	GACGCAATCTAGCGTCGCCAAAGGGTCTTG CATTCCACTTCCCAACGGAAGCGGTGATAC	this study	50
Tn-downstream 2‡	Tn-2F Tn-3R	GCCACACATCACTCCACATAGTTTGCGACA TTCCGCAAAGTTCAGACGGACCTCGATGTG	this study	60
Left terminus	Tn-11F-4F Tn-5R	GAACATATCACCAATAATCTC TTCTTCGCTGAACGACTTTATCCTCGCCAG	this study	60
Right terminus	Tn-2F Tn-3R-2R	GCCACACATCACTCCACATAGTTTGCGACA TTCCGCAAAGTTCAGACGGACCTCGATGTG	this study	60
Flanking region	Tn-11F-4F Tn-3R-2R	GAACATATCACCAATAATCTC TTCCGCAAAGTTCAGACGGACCTCGATGTG	this study	56

* Also used to obtain a specific probe.

† Primers used for inverse PCR.

‡ Primers used for nested PCR.

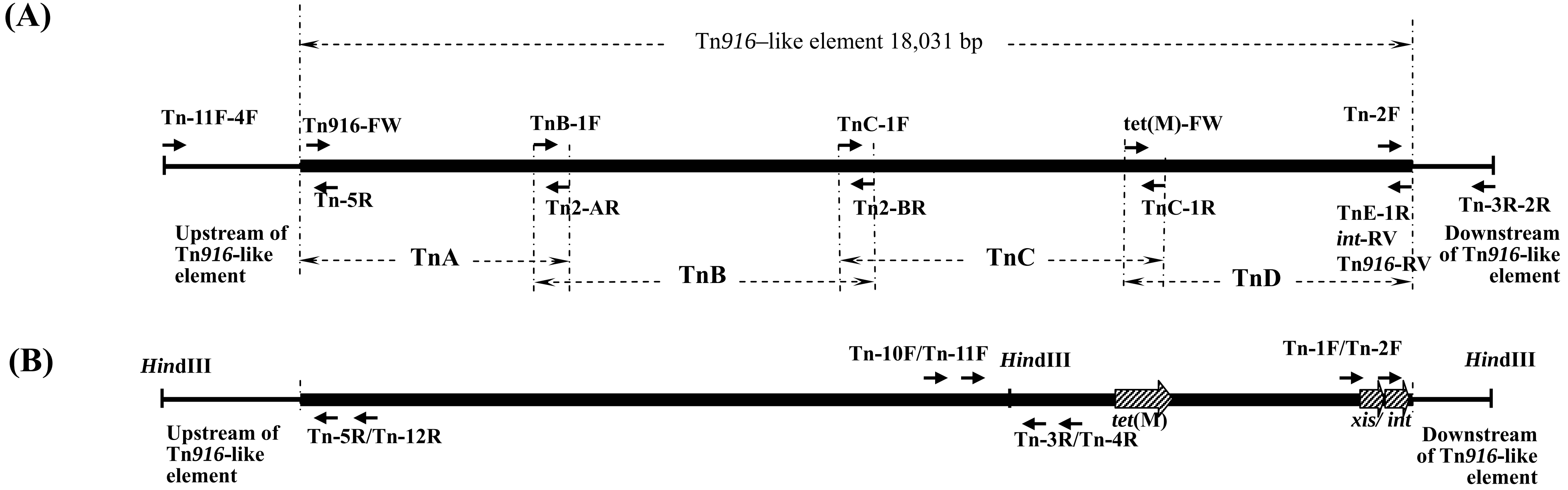

Fig. 4-1. Sequencing strategy of Tn*916*-like element (A) and flanking regions (B). Lengths and directions of solid arrows represent the genes. Arrows show the positions and directions of primers.

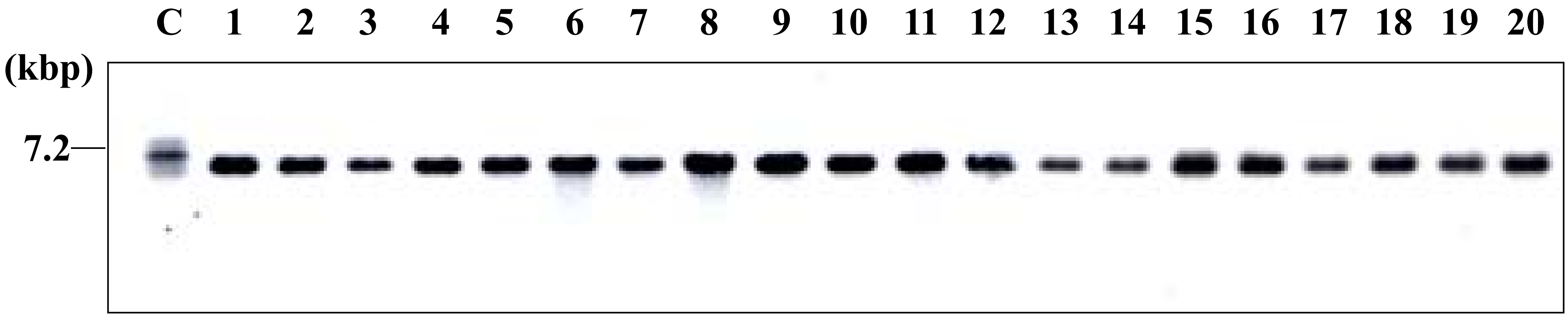

Fig. 4-2. Southern hybridization analysis of Tn*916*-like element. Chromosomal DNAs were digested with *Hin*d III. The digests on the membrane were hybridized with DIG-labeled Tn*916*-like element probe. Lane C: *E. faecalis* CG110 (positive control); Lane 1-20: *S. parauberis* serotype II strains tested in this study.

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S. parauberis   AAAATAGCATAAAAATCTAGTTATCCGCATAAAAACTGGACTTATCACACTTTATCAAGGTCAAAACCACTCAATTTACTACTAATTTACTACTTATGAATGAGCTTTGATACGACGATT 120
S. agalatiae    AAAATAGCATAAAAATCTAGTTATCCGCATAAAAACTGGACTTATCACACTTTATCAAGGTCAAAACCACTCAATTTACTACTAATTTACTACTTATGAATGAGCTTTGATACGACGATT 120
pCF10           AAAATAGCATAAAAATCTAGTTATCCGCATAAAAACTGGACTTATCACACTTTATCAAGGTCAAAACCACTCAATTTACTACTAATTTACTACTTATGAATGAGCTTTGATACGACGATT 120
E. faecalis     AAAATAGCATAAAAATCTAGTTATCCGCATAAAAACTGGACTTATCACACTTTATCAAGGTCAAAACCACTCAATTTACTACTAATTTACTACTTATGAATGAGCTTTGATACGACGATT 120
S. suis         AAAATAGCATAAAAATCTAGTTATCCGCATAAAAACTGGACTTATCACACTTTATCAAGGTCAAAACCACTCAATTTACTACTAATTTACTACTTATGAATGAGCTTTGATACGACGATT 120
                ************************************************************************************************************************

S. parauberis   TATCCTTGAAAAGTGAAGATATAAAGATACTTCCAATAAAATTTGAATATTTAATAGGTAGACACTTCAAAAAATGAGGTGTCTATTTTTTTACCCGATTTTGAAAGGAAGTGAACTTAT 240
S. agalatiae    TATCCTTGAAAAGTGAAGATATAAAGATACTTCCAATAAAATTTGAATATTTAATAGGTAGACACTTCAAAAAATGAGGTGTCTATTTTTTTACCCGATTTTGAAAGGAAGTGAACTTAT 240
pCF10           TATCCTTGAAAAGTGAAGATATAAAGATACTTCCAATAAAATTTGAATATTTAATAGGTAGACACTTCAAAAAATGAGGTGTCTATTTTTTTACCCGATTTTGAAAGGAAGTGAACTTAA 240
E. faecalis     TATCCTTGAAAAGTGAAGATATAAAGATACTTCCAATAAAATTTGAATATTTAATAGGTAGACACTTCAAAAAATGAGGTGTCTATTTTTTTACCCGATTTTGAAAGGAAGTGAACTTAT 240
S. suis         TATCCTTGAAAAGTGAAGATATAAAGATACTTCCAATAAAATTTGAATATTTAATAGGTAGACACTTCAAAAAATGAGGTGTCTATTTTTTTACCCGATTTTGAAAGGAAGTGAACTTAT 240
                ***********************************************************************************************************************

S. parauberis   GAAAACAAAAAATCAAGAATCAAAAGGTCGTTCCCCACTCTTTAAGACCATCAAACATTCATTCAGCCAATAAAAAAGAAAGGATAGGTAAAAATATGGAACTTAAATTTGTGATTCCCA 360
S. agalatiae    GAAAACAAAAAATCAAGAATCAAAAGGTCGTTCCCCACTCTTTAAGACCATCAAACATTCATTCAGCCAATAAAAAAGAAAGGATAGGTAAAAATATGGAACTTAAATTTGTGATTCCCA 360
pCF10           GAAAACAAAAAATCAAGAATCAAAAGGTCGTTCCCCACTCTTTAAGACCATCAAACATTCATTCAGCCAATAAAAAAGAAAGGATAGGTAAAAATATGGAACTTAAATTTGTGATTCCCA 360
E. faecalis     GAAAACAAAAAATCAAGAATCAAAAGGTCGTTCCCCACTCTTTAAGACCATCAAACATTCATTCAGCCAATAAAAAAGAAAGGATAGGTAAAAATATGGAACTTAAATTTGTGATTCCCA 360
S. suis         GAAAACAAAAAATCAAGAATCAAAAGGTCGTTCCCCACTCTTTAAGACCATCAAACATTCATTCAGCCTATAAAAAAGAAAGGATAGGTAAAAATATGGAACTTAAATTTGTGATTCCCA 360
                ******************************************************************** ***************************************************

S. parauberis   ACATGGAAAAAACATTCGGCAATTTAGAATTTGCTGGCGAGGATAAAGTCGTTCAGCGAAGAATCAACGGACGGCTAACTGTCTTATCAAGAAGCTATAATCTCTATTCTGATGTTCAAA 480
S. agalatiae    ACATGGAAAAAACATTCGGCAATTTAGAATTTGCTGGCGAGGATAAAGTCGTTCAGCGAAGAATCAACGGACGGCTAACTGTCTTATCAAGAAGCTATAATCTCTATTCTGATGTTCAAA 480
pCF10           ACATGGAAAAAACATTCGGCAATTTAGAATTTGCTGGCGAGGATAAAGTCGTTCAGCGAAGAATCAACGGACGGCTAACTGTCTTATCAAGAAGCTATAATCTCTATTCTGATGTTCAAA 480
E. faecalis     ACATGGAAAAAACATTCGGCAATTTAGAATTTGCTGGCGAGGATAAAGTCGTTCAGCGAAGAATCAACGGACGGCTAACTGTCTTATCAAGAAGCTATAATCTCTATTCTGATGTTCAAA 480
S. suis         ACATGGAAAAAACATTCGGCAATTTAGAATTTGCTGGCGAGGATAAAGTCGTTCAGCGAAGAATCAACGGACGGCTAACTGTCTTATCAAGAAGCTATAATCTCTATTCTGATGTTCAAA 480
                ************************************************************************************************************************

S. parauberis   GAGCAGATGATATTGTGGTGGTGCTTCCTGCTGAAGCTGGCGAAAAACATTTCGGCTTTGAGGAACGTGTGAAGTTAGTCAATCCACGTATTACCGCAGAGGGCTACAAAATCGGCACTC 600
S. agalatiae    GAGCAGATGATATTGTGGTGGTGCTTCCTGCTGAAGCTGGCGAAAAACATTTCGGCTTTGAGGAACGTGTGAAGTTAGTCAATCCACGTATTACCGCAGAGGGCTACAAAATCGGCACTC 600
pCF10           GAGCAGATGATATTGTGGTGGTGCTTCCTGCTGAAGCTGGCGAAAAACATTTCGGCTTTGAGGAACGTGTGAAGTTAGTCAATCCACGTATTACCGCAGAGGGCTACAAAATCGGCACTC 600
E. faecalis     GAGCAGATGATATTGTGGTGGTGCTTCCTGCTGAAGCTGGCGAAAAACATTTCGGCTTTGAGGAACGTGTGAAGTTAGTCAATCCACGTATTACCGCAGAGGGCTACAAAATCGGCACTC 600
S. suis         GAGCAGATGATATTGTGGTGGTGCTTCCTGCTGAAGCTGGCGAAAAACATTTCGGCTTTGAGGAACGTGTGAAGTTAGTCGATCCACGTATTACCGCAGAGGGCTACAAAATCGGCACTC 600
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S. parauberis   GTGGTTTTACAAATTACCTTTTACATGCTGACGACATGATAAAAGAATAAAGAAAGAGAGGAAAAATGATGAGATTAGCAAATGGCATTGTATTAGATAAAGACACGACTTTTGGAGAAT 720
S. agalatiae    GTGGTTTTACAAATTACCTTTTACATGCTGACGACATGATAAAAGAATAAAGAAAGAGAGGAAAAATGATGAGATTAGCAAATGGCATTGTATTAGATAAAGACACGACTTTTGGAGAAT 720
pCF10           GTGGTTTTACAAATTACCTTTTACATGCTGACGACATGATAAAAGAATAAAGAAAGAGAGGAAAAATGATGAGATTAGCAAATGGCATTGTATTAGATAAAGACACGACTTTTGGAGAAT 720
E. faecalis     GTGGTTTTACAAATTACCTTTTACATGCTGACGACATGATAAAAGAATAAAGAAAGAGAGGAAAAATGATGAGATTAGCAAATGGCATTGTATTAGATAAAGACACGACTTTTGGAGAAT 720
S. suis         GTGGTTTTACAAATTACCTTTTACATGCTGACGACATGATAAAAGAATAAAGAGAGAGAGGAAAAATGATGAGATTAGCAAATGGCATTGTATTAGATAAAGACACGACTTTTGGAGAAT 720
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S. parauberis   TGAAATTCTCTGCTCTACGTCGTGAAGTGAGAATCCAAAATGAAGACGGGTCGGTTTCAGATGAAATCAAGGAACGTACCTATGACTTAAAATCCAAAGGACAAGGACGCATGATTCAAG 840
S. agalatiae    TGAAATTCTCTGCTCTACGTCGTGAAGTGAGAATCCAAAATGAAGACGGGTCGGTTTCAGATGAAATCAAGGAACGTACCTATGACTTAAAATCCAAAGGACAAGGACGCATGATTCAAG 840
pCF10           TGAAATTCTCTGCTCTACGTCGTGAAGTGAGAATCCAAAATGAAGACGGGTCGGTTTCAGATGAAATCAAGGAACGTACCTATGACTTAAAATCCAAAGGACAAGGACGCATGATTCAAG 840
E. faecalis     TGAAATTCTCTGCTCTACGTCGTGAAGTGAGAATCCAAAATGAAGACGGGTCGGTTTCAGATGAAATCAAGGAACGTACCTATGACTTAAAATCCAAAGGACAAGGACGCATGATTCAAG 840
S. suis         TGAAATTCTCTGCTCTACGTCGTGAAGTGAGAATCCAAAATGAAGACGGGTCGGTTTCAGATGAAATCAAGGAACGTACCTATGACTTAAAATCCAAAGGACAAGGACGCATGATTCAAG 840
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S. parauberis   TAAGTATTCCTGCCAGCGTGCCTTTGAAAGAGTTTGATTATAACGCACGGGTGGAACTTATCAATCCCATTGCGGACACCGTTGCTACTGCCACCTATCAAGGAGCAGATGTTGACTGGT 960
S. agalatiae    TAAGTATTCCTGCCAGCGTGCCTTTGAAAGAGTTTGATTATAACGCACGGGTGGAACTTATCAATCCCATTGCGGACACCGTTGCTACTGCCACCTATCAAGGAGCAGATGTTGACTGGT 960
pCF10           TAAGTATTCCTGCCAGCGTGCCTTTGAAAGAGTTTGATTATAACGCACGGGTGGAACTTATCAATCCCATTGCGGACACCGTTGCTACTGCCACCTATCAAGGAGCAGATGTTGACTGGT 960
E. faecalis     TAAGTATTCCTGCCAGCGTGCCTTTGAAAGAGTTTGATTATAACGCACGGGTGGAACTTATCAATCCCATTGCGGACACCGTTGCTACTGCCACCTATCAAGGAGCAGATGTTGACTGGT 960
S. suis         TAAGTATTCCTGCCAGCGTGCCTTTGAAAGAGTTTGATTATAACGCACGGGTGGAACTTATCAATCCCATTGCGGACACCGTTGCTACTGCCACCTATCAAGGAGCAGATGTTGACTGGT 960
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S. parauberis   ATATCAAGGCAGACGATATTGTGCTGACAAAGGATTCTAGTTCATTCAAAGCTCAACCACAAGCAAAGAAAGAACCGACACAAGACAAATAGTCGCTAGGTAGAAAGGAGACTTTTTCGC 1080
S. agalatiae    ATATCAAGGCAGACGATATTGTGCTGACAAAGGATTCTAGTTCATTCAAAGCTCAACCACAAGCAAAGAAAGAACCGACACAAGACAAATAGTCGCTAGGTAGAAAGGAGACTTTTTCGC 1080
pCF10           ATATCAAGGCAGACGATATTGTGCTGACAAAGGATTCTAGTTCATTCAAAGCTCAACCACAAGCAAAGAAAGAACCGACACAAGACAAATAGTCGCTAGGTAGAAAGGAGACTTTTTCGC 1080
E. faecalis     ATATCAAGGCAGACGATATTGTGCTGACAAAGGATTCTAGTTCATTCAAAGCTCAACCACAAGCAAAGAAAGAACCGACACAAGACAAATAGTCGCTAGGTAGAAAGGAGACTTTTTCGC 1080
S. suis         ATATCAAGGCAGACGATATTGTGCTGACAAAGGATTCTAGTTCATTCAAAGCTCAACCACAAGCAAAGAAAGAACCGACACAAGACAAATAGTCGCTAGGTAGAAAGGAGACTTTTTCGC 1080
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S. parauberis   ATGAAACAGCGTGGTAAAAGGATTCGCCCATCTGGTAAAGATTTAGTCTTTCATTTTACGATAGCGTCACTCCTGCCTGTTTTCCTGCTGGTTGTCGGACTGTTTCATGTGAAGACAATC 1200
S. agalatiae    ATGAAACAGCGTGGTAAAAGGATTCGCCCATCTGGTAAAGATTTAGTCTTTCATTTTACGATAGCGTCACTCCTGCCTGTTTTCCTGCTGGTTGTCGGACTGTTTCATGTGAAGACAATC 1200
pCF10           ATGAAACAGCGTGGTAAAAGGATTCGCCCATCTGGTAAAGATTTAGTCTTTCATTTTACGATAGCGTCACTCCTGCCTGTTTTCCTGCTGGTTGTCGGACTGTTTCATGTGAAGACAATC 1200
E. faecalis     ATGAAACAGCGTGGTAAAAGGATTCGCCCATCTGGTAAAGATTTAGTCTTTCATTTTACGATAGCGTCACTCCTGCCTGTTTTCCTGCTGGTTGTCGGACTGTTTCATGTGAAGACAATC 1200
S. suis         ATGAAACAGCGTGGTAAAAGGATTCGCCCATCTGGTAAAGATTTAGTCTTTCATTTTACGATAGCGTCACTCCTGCCTGTTTTCCTGCTGGTTGTCGGACTGTTTCATGTGAAGACAATC 1200
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S. parauberis   CAGCAGATCAACTGGCAGGATTTTAACCTATCACAAGCAGATAAGATTGACATTCCCTATTTAATTATCAGTTTCAGTGTCGCAATTCTTATCTGCTTGCTGGTAGCGTTTGTATTCAAA 1320
S. agalatiae    CAGCAGATCAACTGGCAGGATTTTAACCTATCACAAGCAGATAAGATTGACATTCCCTATTTAATTATCAGTTTCAGTGTCGCAATTCTTATCTGCTTGCTGGTAGCGTTTGTATTCAAA 1320
pCF10           CAGCAGATCAACTGGCAGGATTTTAACCTATCACAAGCAGATAAGATTGACATTCCCTATTTAATTATCAGTTTCAGTGTCGCAATTCTTATCTGCTTGCTGGTAGCGTTTGTATTCAAA 1320
E. faecalis     CAGCAGATCAACTGGCAGGATTTTAACCTATCACAAGCAGATAAGATTGACATTCCCTATTTAATTATCAGTTTCAGTGTCGCAATTCTTATCTGCTTGCTGGTAGCGTTTGTATTCAAA 1320
S. suis         CAGCAGATCAACTGGCAGGATTTTAACCTATCACAAGCAGATAAGATTGACATTCCCTATTTAATTATCAGTTTCAGTGTCGCAATTCTTATCTGCTTGCTGGTAGCGTTTGTATTCAAA 1320
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S. parauberis   CGGGTTCGCTATGATACGGTTAAACAACTTTACCACCGTCAAAAACTGGCAAAGATGATACTTGAAAACAAGTGGTATGAATCTGAACAGGTCAAAACAGAGGGTTTCTTTAAAGATAGT 1440
S. agalatiae    CGGGTTCGCTATGATACGGTTAAACAACTTTACCACCGTCAAAAACTGGCAAAGATGATACTTGAAAACAAGTGGTATGAATCTGAACAGGTCAAAACAGAGGGTTTCTTTAAAGATAGT 1440
pCF10           CGGGTTCGCTATGATACGGTTAAACAACTTTACCACCGTCAAAAACTGGCAAAGATGATACTTGAAAACAAGTGGTATGAATCTGAACAGGTCAAAACAGAGGGTTTCTTTAAAGATAGT 1440
E. faecalis     CGGGTTCGCTATGATACGGTTAAACAACTTTACCACCGTCAAAAACTGGCAAAGATGATACTTGAAAACAAGTGGTATGAATCTGAACAGGTCAAAACAGAGGGTTTCTTTAAAGATAGT 1440
S. suis         CGGGTTCGCTATGATACGGTTAAACAACTTTACCACCGTCAAAAACTGACAAAGATGATACTTGAAAACAAGTGGTATGAATCTGAACAGGTCAAAACAGAGGGTTTCTTTAAAGATAGT 1440
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S. parauberis   GCTGGTCGTACAAAGGAAAAGATAACCTACTTCCCTAAAATGTATTATCGACTTAAAAATGGCTTGATACAGATACGGGTGGAAATCACGCTGGGAAAATATCAAGACCAACTCTTACAC 1560
S. agalatiae    GCTGGTCGTACAAAGGAAAAGATAACCTACTTCCCTAAAATGTATTATCGACTTAAAAATGGCTTGATACAGATACGGGTGGAAATCACGCTGGGAAAATATCAAGACCAACTCTTACAC 1560
pCF10           GCTGGTCGTACAAAGGAAAAGATAACCTACTTCCCTAAAATGTATTATCGACTTAAAAATGGCTTGATACAGATACGGGTGGAAATCACGCTGGGAAAATATCAAGACCAACTCTTACAC 1560
E. faecalis     GCTGGTCGTACAAAGGAAAAGATAACCTACTTCCCTAAAATGTATTATCGACTTAAAAATGGCTTGATACAGATACGGGTGGAAATCACGCTGGGAAAATATCAAGACCAACTCTTACAC 1560
S. suis         GCTGGTCGTACAAAGGAAAAGATAACCTACTTCCCTAAAATGTATTATCGACTTAAAAATGGCTTGATACAGATACGGGTGGAAATCACGCTGGGAAAATATCAAGACCAACTCTTACAC 1560
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S. parauberis   TTGGAAAAGAAATTAGAGAGTGGCTTGTACTGTGAGCTGACGGATAAAGAGTTAAAGGATTCCTATGTGGAATATACTTTGCTCTATGACACCATAGCCAGTCGTATTTCTATTGATGAA 1680
S. agalatiae    TTGGAAAAGAAATTAGAGAGTGGCTTGTACTGTGAGCTGACGGATAAAGAGTTAAAGGATTCCTATGTGGAATATACTTTGCTCTATGACACCATAGCCAGTCGTATTTCTATTGATGAA 1680
pCF10           TTGGAAAAGAAATTAGAGAGTGGCTTGTACTGTGAGCTGACGGATAAAGAGTTAAAGGATTCCTATGTGGAATATACTTTGCTCTATGACACCATAGCCAGTCGTATTTCTATTGATGAA 1680
E. faecalis     TTGGAAAAGAAATTAGAGAGTGGCTTGTACTGTGAGCTGACGGATAAAGAGTTAAAGGATTCCTATGTGGAATATACTTTGCTCTATGACACCATAGCCAGTCGTATTTCTATTGATGAA 1680
S. suis         TTGGAAAAGAAATTAGAGAGTGGCTTGTACTGTGAGCTGACGGATAAAGAGTTAAAGGATTCCTATGTGGAATATACTTTGCTCTATGACACCATAGCCAGTCGTATTTCTATTGATGAA 1680
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S. parauberis   GTAGAAGCTAAAGATGGTAAACTTCGCTTAATGAAAAACGTATGGTGGGAATATGATAAGCTCCCTCATATGTTGATTGCTGGTGGTACAGGTGGCGGTAAAACTTACTTTATACTGACA 1800
S. agalatiae    GTAGAAGCTAAAGATGGTAAACTTCGCTTAATGAAAAACGTATGGTGGGAATATGATAAGCTCCCTCATATGTTGATTGCTGGTGGTACAGGTGGCGGTAAAACTTACTTTATACTGACA 1800
pCF10           GTAGAAGCTAAAGATGGTAAACTTCGCTTAATGAAAAACGTATGGTGGGAATATGATAAGCTCCCTCATATGTTGATTGCTGGTGGTACAGGTGGCGGTAAAACTTACTTTATACTGACA 1800
E. faecalis     GTAGAAGCTAAAGATGGTAAACTTCGCTTAATGAAAAACGTATGGTGGGAATATGATAAGCTCCCTCATATGTTGATTGCTGGTGGTACAGGTGGCGGTAAAACTTACTTTATACTGACA 1800
S. suis         GTAGAAGCTAAAGATGGTAAACCTCGCTTAATGAAAAACGTATGGTGGGAATATGATAAGCTCCCTCATATGTTGATTGCTGGTGGTACAGGTGGCGGTAAAACTTACTTTATACTGACA 1800
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S. parauberis   CTGATTGAAGCCTTGCTTCATACAGATTCAAAACTGTATATTCTTGACCCGAAAAATGCTGATCTTGCGGACTTAGGTTCTGTAATGGCAAATGTCTACTATAGAAAAGAAGACTTGCTT 1920
S. agalatiae    CTGATTGAAGCCTTGCTTCATACAGATTCAAAACTGTATATTCTTGACCCGAAAAATGCTGATCTTGCGGACTTAGGTTCTGTGATGGCAAATGTCTACTATAGAAAAGAAGACTTGCTT 1920
pCF10           CTGATTGAAGCCTTGCTTCATACAGATTCAAAACTGTATATTCTTGACCCGAAAAATGCTGACCTTGCGGACTTAGGTTCTGTGATGGCAAATGTCTACTATAGAAAAGAAGACTTGCTT 1920
E. faecalis     CTGATTGAAGCCTTGCTTCATACAGATTCAAAACTGTATATTCTTGACCCGAAAAATGCTGACCTTGCGGACTTAGGTTCTGTGATGGCAAATGTCTACTATAGAAAAGAAGACTTGCTT 1920
S. suis         CTGATTGAAGCCTTGCTTCATACAGATTCAAAACTGTATATTCTTGACCCGAAAAATGCTGATCTTGCGGACTTAGGTTCTGTGATGGCAAATGTCTACTATAGAAAAGAAGACTTGCTT 1920
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S. parauberis   TCTTGCATTGAAACATTCTATGAAGAAATGATGAAACGTAGTGAGGAAATGAAGCAGATGAAGAACTATAAGACTGGCAAAAATTATGCTTACTTAGGTCTCCCGGCACACTTCTTAATC 2040
S. agalatiae    TCTTGCATTGAAACATTCTATGAAGAAATGATGAAACGTAGTGAGGAAATGAAGCAGATGAAGAACTATAAGACTGGCAAAAATTATGCTTACTTAGGTCTCCCGGCACACTTCTTAATC 2040
pCF10           TCTTGCATTGAAACATTCTATGAAGAAATGATGAAACGTAGTGAGGAAATGAAGCAGATGAAGAACTATAAGACTGGCAAAAATTATGCTTACTTAGGTCTCCCGGCACACTTCTTAATC 2040
E. faecalis     TCTTGCATTGAAACATTCTATGAAGAAATGATGAAACGTAGTGAGGAAATGAAGCAGATGAAGAACTATAAGACTGGCAAAAATTATGCTTACTTAGGTCTCCCGGCACACTTCTTAATC 2040
S. suis         TCTTGCATTGAAACATTCTATGAAGAAATGATGAAACGTAGTGAGGAAATGAAGCAGATGAAGAACTATAAGACTGGCAAAAATTATGCTTACTTAGGTCTCCCGGCACACTTCTTAATC 2040
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S. parauberis   TTTGATGAATACGTCGCTTTCATGGAAATGCTGGGAACAAAAGAAAACACCGCAGTTATGAATAAGCTGAAACAGATTGTCATGTTAGGTCGTCAAGCTGGCTTCTTTCTAATACTGGCT 2160
S. agalatiae    TTTGATGAATACGTCGCTTTCATGGAAATGCTGGGAACAAAAGAAAACACCGCAGTTATGAATAAGCTGAAACAGATTGTCATGTTAGGTCGTCAAGCTGGCTTCTTTCTAATACTGGCT 2160
pCF10           TTTGATGAATACGTCGCTTTCATGGAAATGCTGGGAACAAAAGAAAACACCGCAGTTATGAATAAGCTGAAACAGATTGTCATGTTAGGTCGTCAAGCTGGCTTCTTTCTAATACTGGCT 2160
E. faecalis     TTTGATGAATACGTCGCTTTCATGGAAATGCTGGGAACAAAAGAAAACACCGCAGTTATGAATAAGCTGAAACAGATTGTCATGTTAGGTCGTCAAGCTGGCTTCTTTCTAATACTGGCT 2160
S. suis         TTTGATGAATACGTCGCTTTCATGGAAATGCTGGGAACAAAAGAAAACACCGCAGTTATGAATAAGCTGAAACAGATTGTCATGTTAGGTCGTCAAGCTGGCTTCTTTCTAATACTGGCT 2160
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S. parauberis   TGTCAACGTCCAGACGCAAAATATTTAGGCGACGGAATCCGTGATCAGTTTAATTTCAGAGTGGCTTTAGGTCGTATGTCTGAAATGGGCTATGGCATGATGTTTGGCAGTGACGTACAA 2280
S. agalatiae    TGTCAACGTCCAGACGCAAAATATTTAGGCGACGGAATCCGTGATCAGTTTAATTTCAGAGTGGCTTTAGGTCGTATGTCTGAAATGGGCTATGGCATGATGTTTGGCAGTGACGTACAA 2280
pCF10           TGTCAACGTCCAGACGCAAAATATTTAGGCGACGGAATCCGTGATCAGTTTAATTTCAGAGTGGCTTTAGGTCGTATGTCTGAAATGGGCTATGGCATGATGTTTGGCAGTGACGTACAA 2280
E. faecalis     TGTCAACGTCCAGACGCAAAATATTTAGGCGACGGAATCCGTGATCAGTTTAATTTCAGAGTGGCTTTAGGTCGTATGTCTGAAATGGGCTATGGCATGATGTTTGGCAGTGACGTACAA 2280
S. suis         TGTCAACGTCCAGACGCAAAATATTTAGGCGGCGGAATCCGTGATCAGTTTAATTTCAGAGTGGCTTTAGGTCGTATGTCTGAAATGGGCTATGGCATGATGTTTGGCAGTGACGTACAA 2280
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S. parauberis   AAGGATTTCTTCTTAAAGCGAATCAAAGGTCGTGGCTATGTTGATGTAGGAACAAGTGTCATATCAGAGTTTTATACTCCCCTTGTACCAAAAGGATATGATTTCTTGGAGGAAATTAAA 2400
S. agalatiae    AAGGATTTCTTCTTAAAGCGAATCAAAGGTCGTGGCTATGTTGATGTAGGAACAAGTGTCATATCAGAGTTTTATACTCCCCTTGTACCAAAAGGATATGATTTCTTGGAGGAAATTAAA 2400
pCF10           AAGGATTTCTTCTTAAAGCGAATCAAAGGTCGTGGCTATGTTGATGTAGGAACAAGTGTCATATCAGAGTTTTATACTCCCCTTGTACCAAAAGGATATGATTTCTTGGAGGAAATTAAA 2400
E. faecalis     AAGGATTTCTTCTTAAAGCGAATCAAAGGTCGTGGCTATGTTGATGTAGGAACAAGTGTCATATCAGAGTTTTATACTCCCCTTGTACCAAAAGGATATGATTTCTTGGAGGAAATTAAA 2400
S. suis         AAGGATTTCTTCTTAAAGCGAATCAAAGGTCGTGGCTATGTTGATGTAGGAACAAGTGTCATATCAGAGTTTTATACTCCCCTTGTACCAAAAGGATATGATTTCTTGGAGGAAATTAAA 2400
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S. parauberis   AAGTTATCCAACAGCAGACAGTCCACGCAGGCGACGTGCGAAGCGGAAGTCGCAGGTGTGGACTGATCTTGCTGGCTGGTGTGGCAATAGCCACGCCAGCACTTAACCCCCCGTATCTAA 2520
S. agalatiae    AAGTTATCCAACAGCAGACAGTCCACGCAGGCGACGTGCGAAGCGGAAGTCGCAGGTGTGGACTGATCTTGCTGGCTGGTGTGGCAATAGCCACGCCAGCACTTAACCCCCCGTATCTAA 2520
pCF10           AAGTTATCCAACAGCAGACAGTCCACGCAGGCGACGTGCGAAGCGGAAGTCGCAGGTGTGGACTGATCTTGCTGGCTGGTGTGGCAATAGCCACGCCAGCACTTAACCCCCCGTATCTAA 2520
E. faecalis     AAGTTATCCAACAGCAGACAGTCCACGCAGGCGACGTGCGAAGCGGAAGTCGCAGGTGTGGACTGATCTTGCTGGCTGGTGTGGCAATAGCCACGCCAGCACTTAACCCCCCGTATCTAA 2520
S. suis         AAGTTATCCAACAGCAGACAGTCCACGCAGGCGACGTGCGAAGCGGAAGTCGCAGGTGTGGACTGATCTTGCTGGCTGGTGTGGCAATAGCCACGCCAGCACTTAACCCCCCGTATCTAA 2520
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S. parauberis   CAGGGGGGTACAAATCGACAGGAAACAGTCAAAAAAACATTAGAAAATCCTTTGGTTACAAGGGATTTACAAAATTTCAGCGTATGTCAAATGGGCTTTAAAAGTTGACATACG-CCTTT 2639
S. agalatiae    CAGGGGGGTACAAATCGACAGGAAACAGTCAAAAAAACATTAGAAAATCCTTTGGTTACAAGGGATTTACAAAATTTCAGCGTATGTCAAATGGGCTTTAAAAGTTGACATACG-CCTTT 2639
pCF10           CAGGGGGGTACAAATCGACAGGAAACAGTCAAAAAAACATTAGAAAATCCTTTGGTTACAAGGGATTTACAAAATTTCAGCGTATGTCAAATGGGCTTTAAAAGTTGACATACG-CCTTT 2639
E. faecalis     CAGGGGGGTACAAATCGACAGGAAACAGTCAAAAAAACATTAGAAAATCCTTTGGTTACAAGGGATTTACAAAATTTCAGCGTATGTCAAATGGGCTTTAAAAGTTGACATACGGCCTTT 2640
S. suis         CAGGGGGGTACAAATCGACAGGAAACAGTCAAAAAAACATTAGAAAATCCTTTGGTTACAAGGGATTTACAAAATTTCAGCGTATGTCAAATGGGCTTTAAAAGTTGACATACG-CCTTT 2639
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S. parauberis    TTGATTGGAGGGATTTTTACTGAATGAACAAACTTGGTTACAGCATTTAAAAGAAAAACGCTTGGCTTATGGACTATCTCAAAACCGTTTAGCTGTTGCGACTGGTATTACAAGGCAGTA 2759
S. agalatiae     TTGATTGGAGGGATTTTTACTGAATGAACAAACTTGGTTACAGCATTTAAAAGAAAAACGCTTGGCTTATGGACTATCTCAAAACCGTTTAGCTGTTGCGACTGGTATTACAAGGCAGTA 2759
pCF10            TTGATTGGAGGGATTTTTACTGAATGAACAAACTTGGTTACAGCATTTAAAAGAAAAACGCTTGGCTTATGGACTATCTCAAAACCGTTTAGCTGTTGCGACTGGTATTACAAGGCAGTA 2759
E. faecalis      TTGATTGGAGGGATTTTTACTGAATGAACAAACTTGGTTACAGCATTTAAAAGAAAAACGCTTGGCTTATGGACTATCTCAAAACCGTTTAGCTGTTGCGACTGGTATTACAAGGCAGTA 2760
S. suis          TTGATTGGAGGGATTTTTACTGAATGAACAAACTTGGTTACAGCATTTAAAAGAAAAACGCTTGGCTTATGGACTATCTCAAAACCGTTTAGCTGTTGCGACTGGTATTACAAGGCAGTA 2759
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S. parauberis    TCTAAGCGATATTGAAACAGGAAAAGTCAAGCCATCAGAGGATTTACAGCAGTCCCTTTGGGAAGCTCTGGAACGCTTCAATCCCGACGCTCCCCTTGAAATGCTGTTTGATTATGTAAG 2879
S. agalatiae     TCTAAGCGATATTGAAACAGGAAAAGTCAAGCCATCAGAGGATTTACAGCAGTCCCTTTGGGAAGCTCTGGAACGCTTCAATCCCGACGCTCCCCTTGAAATGCTGTTTGATTATGTAAG 2879
pCF10            TCTAAGCGATATTGAAACAGGAAAAGTCAAGCCATCAGAGGATTTACAGCAGTCCCTTTGGGAAGCTCTGGAACGCTTCAATCCCGACGCTCCCCTTGAAATGCTGTTTGATTATGTAAG 2879
E. faecalis      TCTAAGCGATATTGAAACAGGAAAAGTCAAGCCATCAGAGGATTTACAGCAGTCCCTTTGGGAAGCTCTGGAACGCTTCAATCCCGACGCTCCCCTTGAAATGCTGTTTGATTATGTAAG 2880
S. suis          TCTAAGCGATATTGAAACAGGAAAAGTCAAGCCATCAGAGGATTTACAGCAGTCCCTTTGGGAAGCTCTGGAACGCTTCAATCCCGACGCTCCCCTTGAAATGCTGTTTGATTATGTAAG 2879
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S. parauberis    AATTCGCTTTCCGACAACAGACGTACAGCAGGTGGTCGAAAACATCTTACAACTGAAACTGTCCTATTTTCTTCATGAGGACTATGGTTTCTATTCTTATTCAGAGCATTATGCTTTAGG 2999
S. agalatiae     AATTCGCTTTCCGACAACAGACGTACAGCAGGTGGTCGAAAACATCTTACAACTGAAACTGTCCTATTTTCTTCATGAGGACTATGGTTTCTATTCTTATTCAGAGCATTATGCTTTAGG 2999
pCF10            GATTCGCTTTCCGACAACAGACGTACAGCAGGTGGTCGAAAACATCTTACAACTGAAACTGTCCTATTTTCTTCATGAGGACTATGGTTTCTATTCTTATTCAGAGCATTATGCTTTAGG 2999
E. faecalis      GATTCGCTTTCCGACAACAGACGTACAGCAGGTGGTCGAAAACATCTTACAACTGAAACTGTCCTATTTTCTTCATGAGGACTATGGTTTCTATTCTTATTCAGAGCATTATGCTTTAGG 3000
S. suis          AATTCGCTTTCCGACAACAGACGTACAGCAGGTAGTCGAAAACATCTTACAACTGAAACTGTCCTATTTTCTTCATGAGGACTATGGTTTCTATTCTTATTCAGAGCATTATGCTTTAGG 2999
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S. parauberis    CGACATATTCGTCCTTTGCTCCCATGAACTGGACAAAGGAGTTCTGGTGGAATTGAAAGGTCGTGGGTGCAGACAATTTGAAAGCTATCTTCTGGCACAACAAAGAAGCTGGTATGAGTT 3119
S. agalatiae     CGACATATTCGTCCTTTGCTCCCATGAACTGGACAAAGGAGTTCTGGTGGAATTGAAAGGTCGTGGGTGCAGACAATTTGAAAGCTATCTTCTGGCACAACAAAGAAGCTGGTATGAGTT 3119
pCF10            CGACATATTCGTCCTTTGCTCCCATGAACTGGACAAAGGAGTTCTGGTGGAATTGAAAGGTCGTGGGTGCAGACAATTTGAAAGCTATCTTCTGGCACAACAAAGAAGCTGGTATGAGTT 3119
E. faecalis      CGACATATTCGTCCTTTGCTCCCATGAACTGGACAAAGGAGTTCTGGTGGAATTGAAAGGTCGTGGGTGCAGACAATTTGAAAGCTATCTTCTGGCACAACAAAGAAGCTGGTATGAGTT 3120
S. suis          CGACATATTCGTCCTTTGCTCCCATGAACTGGACAAAGGAGTTCTGGTGGAATTGAAAGGTCGTGGGTGCAGACAATTTGAAAGCTATCTTCTGGCACAACAAAGAAGCTGGTATGAGTT 3119
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S. parauberis    CTTTATGGACGTTTTGGTGGCTGGCGGTGTGATGAAACGCCTTGACCTTGCCATTAACGATAAGACAGGGATTTTAAATATCCCTGTACTCACTGAAAAGTGCCAACAGGAAGAATGTAT 3239
S. agalatiae     CTTTATGGACGTTTTGGTGGCTGGCGGTGTGATGAAACGCCTTGACCTTGCCATTAACGATAAGACAGGGATTTTAAATATCCCTGTACTCACTGAAAAGTGCCAACAGGAAGAATGTAT 3239
pCF10            CTTTATGGACGTTTTGGTGGCTGGCGGTGTGATGAAACGCCTTGACCTTGCCATTAACGATAAGACAGGGATTTTGAATATCCCTGTACTCACTGAAAAGTGCCAACAGGAAGAATGTAT 3239
E. faecalis      CTTTATGGACGTTTTGGTGGCTGGCGGTGTGATGAAACGCCTTGACCTTGCCATTAACGATAAGACAGGGATTTTGAATATCCCTGTACTCACTGAAAAGTGCCAACAGGAAGAATGTAT 3240
S. suis          CTTTATGGACGTTTTGGTGGCTGGCGGTGTGATGAAACGCCTTGACCTTGCCATTAACGATAAGACAGGGATTTTAAATATCCCTGTACTCACTGAAAAGTGCCAACAGGAAGAATGTAT 3239
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S. parauberis    CTCCGTCTTCCGCAGTTTTAAAAGCTATCGCAGTGGCGAACTGGTACGCAAAGAGGAAAAGGAATGTATGGGAAACACCCTCTATATCGGTTCATTACAAAGTGAAGTTTATTTCTGTAT 3359
S. agalatiae     CTCCGTCTTCCGCAGTTTTAAAAGCTATCGCAGTGGCGAACTGGTACGCAAAGAGGAAAAGGAATGTATGGGAAACACCCTCTATATCGGTTCATTACAAAGTGAAGTTTATTTCTGTAT 3359
pCF10            CTCCGTCTTCCGCAGTTTTAAAAGCTATCGCAGTGGCGAACTGGTACGCAAAGAGGAAAAGGAATGTATGGGAAACACCCTCTATATCGGTTCATTACAAAGTGAAGTTTATTTCTGTAT 3359
E. faecalis      CTCCGTCTTCCGCAGTTTTAAAAGCTATCGCAGTGGCGAACTGGTACGCAAAGAGGAAAAGGAATGTATGGGAAACACCCTCTATATCGGTTCATTACAAAGTGAAGTTTATTTCTGTAT 3360
S. suis          CTCCGTCTTCCGCAGTTTTAAAAGCTATCGCAGTGGCGAACTGGTACGCAAAGAGGAAAAGGAATGTATGGGAAACACCCTCTATATCGGTTCATTACAAAGTGAAGTTTATTTCTGTAT 3359
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S. parauberis    CTATGAAAAGGACTACGAGCAGTACAAGAAAAATGATATTCCCATTGAAGACGCAGAAGTAAAAAACCGTTTTGAGATTCGATTGAAAAATGAGCGTGCCTATTATGCAGTCCGTGATTT 3479
S. agalatiae     CTATGAAAAGGACTACGAGCAGTACAAGAAAAATGATATTCCCATTGAAGACGCAGAAGTAAAAAACCGTTTTGAGATTCGATTGAAAAATGAGCGTGCCTATTATGCAGTCCGTGATTT 3479
pCF10            CTATGAAAAGGACTACGAGCAGTACAAGAAAAATGATATTCCCATTGAAGACGCAGAAGTAAAAAACCGTTTTGAGATTCGATTGAAAAATGAGCGTGCCTATTATGCAGTCCGTGATTT 3479
E. faecalis      CTATGAAAAGGACTACGAGCAGTACAAGAAAAATGATATTCCCATTGAAGACGCAGAAGTAAAAAACCGTTTTGAGATTCGATTGAAAAATGAGCGTGCCTATTATGCAGTCCGTGATTT 3480
S. suis          CTATGAAAAGGACTACGAGCAGTACAAGAAAAATGATATTCCCATTGAAGACGCAGAAGTAAAAAACCGTTTTGAGATTCGATTGAAAAATGAGCGTGCCTATTATGCAGTCCGTGATTT 3479
                 ************************************************************************************************************************

S. parauberis    ACTCGTCTATGACAATCCAGAGCATACCGCCTTTAAAATTATCAATCGGTATATCCGTTTTGTAGATAAAGACGATTCCAAACCTCGTTCTGATTGGAAACTGAATGAAGAATGGGCTTG 3599
S. agalatiae     ACTCGTCTATGACAATCCAGAGCATACCGCCTTTAAAATTATCAATCGGTATATCCGTTTTGTAGATAAAGACGATTCCAAACCTCGTTCTGATTGGAAACTGAATGAAGAATGGGCTTG 3599
pCF10            ACTCGTCTATGACAATCCAGAGCATACCGCCTTTAAAATTATCAATCGGTATATCCGTTTTGTAGATAAAGACGATTCCAAACCTCGTTCTGATTGGAAACTGAATGAAGAATGGGCTTG 3599
E. faecalis      ACTCGTCTATGACAATCCAGAGCATACCGCCTTTAAAATTATCAATCGGTATATCCGTTTTGTAGATAAAGACGATTCCAAACCTCGTTCTGATTGGAAACTGAATGAAGAATGGGCTTG 3600
S. suis          ACTCGTCTATGACAATCCAGAGCATACCGCCTTTAAAATTATCAATCGGTATATCCGTTTTGTAGATAAAGACGATTCCAAACCTCGTTCTGATTGGAAACTGAATGAAGAATGGGCTTG 3599
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S. parauberis    GTTTATTGGGAACAATCGTGAACGATTAAAACTAACCACAAAACCAGAGCCTTACTCCTTCCAAAGGACGCTGAACTGGCTATCTCATCAAGTTGCCCCGACCTTAAAGGTTGCGATTAA 3719
S. agalatiae     GTTTATTGGGAACAATCGTGAACGATTAAAACTAACCACAAAACCAGAGCCTTACTCCTTCCAAAGGACGCTGAACTGGCTATCTCATCAAGTTGCCCCGACCTTAAAGGTTGCGATTAA 3719
pCF10            GTTTATTGGGAACAATCGTGAACGATTAAAACTAACCACAAAACCAGAGCCTTACTCCTTCCAAAGGACGCTGAACTGGCTATCTCATCAAGTTGCCCCGACCTTAAAGGTTGCGATTAA 3719
E. faecalis      GTTTATTGGGAACAATCGTGAACGATTAAAACTAACCACAAAACCAGAGCCTTACTCCTTCCAAAGGACGCTGAACTGGCTATCTCATCAAGTTGCCCCGACCTTAAAGGTTGCGATTAA 3720
S. suis          GTTTATTGGGAACAATCGTGAACGATTAAAACTAACCACAAAACCAGAGCCTTACTCCTTCCAAAGGACGCTGAACTGGCTATCTCATCAAGTTGCCCCGACCTTAAAGGTTGCGATTAA 3719
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S. parauberis    ACTTGATGAAATCAACCAGACGCAGGTTGTAAAAGACATTCTCGACCATGCGAAACTGACAGACCGACACAAGCAGATTTTGAAGCAACAGTCAGTAAAAGAACAGGACGTGATAACAAC 3839
S. agalatiae     ACTTGATGAAATCAACCAGACGCAGGTTGTAAAAGACATTCTCGACCATGCGAAACTGACAGACCGACACAAGCAGATTTTGAAGCAACAGTCAGTAAAAGAACAGGACGTGATAACAAC 3839
pCF10            ACTTGATGAAATCAACCAGACGCAGGTTGTAAAAGACATTCTCGACCATGCGAAACTGACAGACCGACACAAGCAGATTTTGAAGCAACAGTCAGTAAAAGAACAGGACGTGATAACAAC 3839
E. faecalis      ACTTGATGAAATCAACCAGACGCAGGTTGTAAAAGACATTCTCGACCATGCGAAACTGACAGACCGACACAAGCAGATTTTGAAGCAACAGTCAGTAAAAGAACAGGACGTGATAACAAC 3840
S. suis          ACTTGATGAAATCAACCAGACGCAGGTTGTAAAAGACATTCTCGACCATGCGAAACTGACAGACCGACACAAGCAGATTTTGAAGCAACAGTCAGTAAAAGAACAGGACGTGATAACAAC 3839
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S. parauberis    AAAAAAATAACTCAAATACAAATTCATTGAATATAGAGAGGAGAACATTTTTATGAATTTTGGACAAAACCTTTATAACTGGTTTCTATCAAACGCTCAATCACTGGTGCTTTTAGCAAT 3959
S. agalatiae     AAAAAAATAACTCAAATACAAATTCATTGAATATAGAGAGGAGAACATTTTTATGAATTTTGGACAAAACCTTTATAACTGGTTTCTATCAAACGCTCAATCACTGGTGCTTTTAGCAAT 3959
pCF10            AAAAAAATAACTCAAATACAAATTCATTGAATATAGAGAGGAGAACATTTTTATGAATTTTGGACAAAACCTTTATAACTGGTTTCTATCAAACGCTCAATCACTGGTGCTTTTAGCAAT 3959
E. faecalis      AAAAAAATAACTCAAATACAAATTCATTGAATATAGAGAGGAGAACATTTTTATGAATTTTGGACAAAACCTTTATAACTGGTTTCTATCAAACGCTCAATCACTGGTGCTTTTAGCAAT 3960
S. suis          AAAAAAATAACTCAAATACAAATTCATTGAATATAGAGAGGAGAACATTTTTATGAATTTTGGACAAAACCTTTATAACTGGTTTCTATCAAACGCTCAATCACTGGTGCTTTTAGCAAT 3959
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S. parauberis   CGTTGTGATTGGCTTGTATCTTGGCTTCAAGCGTGAGTTTAGCAAACTGATTGGCTTTTTAATTATTGCGATTATTGCGGTTGGCTTAGTCTTCAACGCTGCTGGAGTAAAAGACATTTT 4079
S. agalatiae    CGTTGTGATTGGCTTGTATCTTGGCTTCAAGCGTGAGTTTAGCAAACTGATTGGCTTTTTAATTATTGCGATTATTGCGGTTGGCTTAGTCTTCAACGCTGCTGGAGTAAAAGACATTTT 4079
pCF10           CGTTGTGATTGGCTTGTATCTTGGCTTCAAGCGTGAGTTTAGCAAACTGATTGGCTTTTTAATTATTGCGATTATTGCGGTTGGCTTAGTCTTCAACGCTGCTGGAGTAAAAGACATTTT 4079
E. faecalis     CGTTGTGATTGGCTTGTATCTTGGCTTCAAGCGTGAGTTTAGCAAACTGATTGGCTTTTTAATTATTGCGATTATTGCGGTTGGCTTAGTCTTCAACGCTGCTGGAGTAAAAGACATTTT 4080
S. suis         CGTTGTGATTGGCTTGTATCTTGGCTTCAAGCGTGAGTTTAGCAAACTGATTGGCTTTTTAATTATTGCGATTATTGCGGTTGGCTTAGTCTTCAACGCTGCTGGAGTAAAAGACATTTT 4079
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S. parauberis   ACTAGAGCTATTCAATCGCATTATTGGTGCTTAAATAAAACCGTTCTTTTGTGGAATATAAGTGGTTTTCTTATGTTCCGCAAAGGAATGGTACACCAAACGAAGTGCGGTAGGGATTTT 4199
S. agalatiae    ACTAGAGCTATTCAATCGCATTATTGGTGCTTAAATAAAACCGTTCTTTTGTGGAATATAAGTGGTTTTCTTATGTTCCGCAAAGGAATGGTACACCAAACGAAGTGCGGTAGGGATTTT 4199
pCF10           ACTAGAGCTATTCAATCGCATTATTGGTGCTTAAATAAAACCGTTCTTTTGTGGAATATAAGTGGTTTTCTTATGTTCCGCAAAGGAATGGTACACCAAACGAAGTGCGGTAGGGATTTT 4199
E. faecalis     ACTAGAGCTATTCAATCGCATTATTGGTGCTTAAATAAAACCGTTCTTTTGTGGAATATAAGTGGTTTTCTTATGTTCCGCAAAGGAATGGTACACCAAACGAAGTGCGGTAGGGATTTT 4200
S. suis         ACTAGAGCTATTCAATCGCATTATTGGTGCTTAAATAAAACCGTTCTTTTGTGGAATATAAGTGGTTTTCTTATGTTCCGCAAAGGAATGGTACACCAAACGAAGTGCGGTAGGGATTTT 4199
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S. parauberis   TGAATCTCTACAAAGAAAGGACGTGAATATATGGACGATATGCAAGTCTATATTGCGAATTTAGGCAAATACAATGAGGGCGAATTGGTCGGTGCGTGGTTTACCTTTCCCATTGACTTT 4319
S. agalatiae    TGAATCTCTACAAAGAAAGGACGTGAATATATGGACGATATGCAAGTCTATATTGCGAATTTAGGCAAATACAATGAGGGCGAATTGGTCGGTGCGTGGTTTACCTTTCCCATTGACTTT 4319
pCF10           TGAATCTCTACAAAGAAAGGACGTGAATATATGGACGATATGCAAGTCTATATTGCGAATTTAGGCAAATACAATGAGGGCGAATTGGTCGGTGCGTGGTTTACCTTTCCCATTGACTTT 4319
E. faecalis     TGAATCTCTACAAAGAAAGGACGTGAATATATGGACGATATGCAAGTCTATATTGCGAATTTAGGCAAATACAATGAGGGCGAATTGGTCGGTGCGTGGTTTACCTTTCCCATTGACTTT 4320
S. suis         TGAATCTCTACAAAGAAAGGACGTGAATATATGGACGATATGCAAGTCTATATTGCGAATTTAGGCAAATACAATGAGGGCGAATTGGTCGGTGCGTGGTTTACCTTTCCCATTGACTTT 4319
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S. parauberis   GAGGAAGTCAAAGAGAAAATCGGCTTGAATGATGAATATGAGGAATACGCCATTCATGACTACGAGTTACCCTTTACGGTTGACGAATACACTTCCATTGGCGAACTCAATCGACTATGG 4439
S. agalatiae    GAGGAAGTCAAAGAGAAAATCGGCTTGAATGATGAATATGAGGAATACGCCATTCATGACTACGAGTTACCCTTTACGGTTGACGAATACACTTCCATTGGCGAACTCAATCGACTATGG 4439
pCF10           GAGGAAGTCAAAGAGAAAATCGGCTTGAATGATGAATATGAGGAATACGCCATTCATGACTACGAGTTACCCTTTACGGTTGACGAATACACTTCCATTGGCGAACTCAATCGACTATGG 4439
E. faecalis     GAGGAAGTCAAAGAGAAAATCGGCTTGAATGATGAATATGAGGAATACGCCATTCATGACTACGAGTTACCCTTTACGGTTGACGAATACACTTCCATTGGCGAACTCAATCGACTATGG 4440
S. suis         GAGGAAGTCAAAGAGAAAATCGGCTTGAATGATGAATATGAGGAATACGCCATTCATGACTACGAGTTACCCTTTACGGTTGACGAATACACTTCCATTGGCGAACTCAATCGACTATGG 4439
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S. parauberis   GAAATGGTATCGGAATTACCCGAAGAATTACAATCGGAGCTATCTGCTCTGCTCACTCATTTTTCAAGCATTGAAGAACTAAGCGAACATCAAGAGGATATTATCATTCATTCCGATTGT 4559
S. agalatiae    GAAATGGTATCGGAATTACCCGAAGAATTACAATCGGAGCTATCTGCTCTGCTCACTCATTTTTCAAGCATTGAAGAACTAAGCGAACATCAAGAGGATATTATCATTCATTCCGATTGT 4559
pCF10           GAAATGGTATCGGAATTACCCGAAGAATTACAATCGGAGCTATCTGCTCTGCTCACTCATTTTTCAAGCATTGAAGAACTAAGCGAACATCAAGAGGATATTATCATTCATTCCGATTGT 4559
E. faecalis     GAAATGGTATCGGAATTACCCGAAGAATTACAATCGGAGCTATCTGCTCTGCTCACTCATTTTTCAAGCATTGAAGAACTAAGCGAACATCAAGAGGATATTATCATTCATTCCGATTGT 4560
S. suis         GAAATGGTATCGGAATTACCCGAAGAATTACAATCGGAGCTATCTGCTCTGCTCACTCATTTTTCAAGCATTGAAGAACTAAGCGAACATCAAGAGGATATTATCATTCATTCCGATTGT 4559
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S. parauberis   GATGATATGTATGACGTGGCACGCTACTACATTGAAGAAACGGGTGCTTTAGGCGAAGTACCAGCTAGTCTTCAAAACTATATTGATTATCAAGCCTATGGTCGGGATTTAGACCTTTCA 4679
S. agalatiae    GATGATATGTATGACGTGGCACGCTACTACATTGAAGAAACGGGTGCTTTAGGCGAAGTACCAGCTAGTCTTCAAAACTATATTGATTATCAAGCCTATGGTCGGGATTTAGACCTTTCA 4679
pCF10           GATGATATGTATGACGTGGCACGCTACTACATTGAAGAAACGGGTGCTTTAGGCGAAGTACCAGCTAGTCTTCAAAACTATATTGATTATCAAGCCTATGGTCGGGATTTAGACCTTTCA 4679
E. faecalis     GATGATATGTATGACGTGGCACGCTACTACATTGAAGAAACGGGTGCTTTAGGCGAAGTACCAGCTAGTCTTCAAAACTATATTGATTATCAAGCCTATGGTCGGGATTTAGACCTTTCA 4680
S. suis         GATGATATGTATGACGTGGCACGCTACTACATTGAAGAAACGGGTGCTTTAGGCGAAGTACCAGCTAGTCTTCAAAACTATATTGATTATCAAGCCTATGGTCGGGATTTAGACCTTTCA 4679
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S. parauberis   GGAACGTTTATCTCAACCAATCATGGGATTTTTGAAATCGTCTATTAAAACTGTCGGTACATTACTACTGGCAGATTTTCTATTTTACGGGGTGGCTCAATCAGCTACCCCTATTTTTTA 4799
S. agalatiae    GGAACGTTTATCTCAACCAATCATGGGATTTTTGAAATCGTCTATTAAATCTGTCGGTACATTACTACTGGCAGATTTTCTATTTTACGGGGTGGCTCAATCAGCTACCCCTATTTTTTA 4799
pCF10           GGAACGTTTATCTCAACCAATCATGGGATTTTTGAAATCGTCTATTAAATCTGTCGGTACATTACTACTGGCAGATTTTCTATTTTACGGGGTGGCTCAATCAGCTACCCCTATTTTTTA 4799
E. faecalis     GGAACGTTTATCTCAACCAATCATGGGATTTTTGAAATCGTCTATTAAATCTGTCGGTACATTACTACTGGCAGATTTTCTATTTTACGGGGTGGCTCAATCAGCTACCCCTATTTTTTA 4800
S. suis         GGAACGTTTATCTCAACCAATCATGGGATTTTTGAAATCGTCTATTAAATCTGTCGGTACATTACTACTGGCAGATTTTCTATTTTACGGGGTGGCTCAATCAGCTACCCCTATTTTTTA 4799
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S. parauberis   TGAAAGGATTGATTACATGAAGAAAATACGAAGCTATACCAGTATCTGGTCTGTGGAAAAGGTACTGTATTCTATCAATGATTTTAGACTTCCGTTTCCCATAACCTTTACGCAAATGAC 4919
S. agalatiae    TGAAAGGATTGATTACATGAAGAAAATACGAAGCTATACCAGTATCTGGTCTGTGGAAAAGGTACTGTATTCTATCAATGATTTTAGACTTCCGTTTCCCATAACCTTTACGCAAATGAC 4919
pCF10           TGAAAGGATTGATTACATGAAGAAAATACGAAGCTATACCAGTATCTGGTCTGTGGAAAAGGTACTGTATTCTATCAATGATTTTAGACTTCCGTTTCCCATAACCTTTACGCAAATGAC 4919
E. faecalis     TGAAAGGATTGATTACATGAAGAAAATACGAAGCTATACCAGTATCTGGTCTGTGGAAAAGGTACTGTATTCTATCAATGATTTTAGACTTCCGTTTCCCATAACCTTTACGCAAATGAC 4920
S. suis         TGAAAGGATTGATTACATGAAGAAAATACGAAGCTATGCCAGTATCTGGTCTGTGGAAAAGGTACTGTATTCTATCAATGATTTTAGACTTCCGTTTCCCATAACCTTTACGCAAACGAC 4919
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S. parauberis   ATGGTTTGTCGTGTCACTCTTTGCAGTGATGATACTTGGCAACTTGCCCCCTCTTTCCATGATAGAGGGAGCATTTCTCAAATACTTTGGGATTCCTGTGGCTTTCACATGGTTTATGTC 5039
S. agalatiae    ATGGTTTGTCGTGTCACTCTTTGCAGTGATGATACTTGGCAACTTGCCCCCTCTTTCCATGATAGAGGGAGCATTTCTCAAATACTTTGGGATTCCTGTGGCTTTCACATGGTTTATGTC 5039
pCF10           ATGGTTTGTCGTGTCACTCTTTGCAGTGATGATACTTGGCAACTTGCCCCCTCTTTCCATGATAGAGGGAGCATTTCTCAAATACTTTGGGATTCCTGTGGCTTTCACATGGTTTATGTC 5039
E. faecalis     ATGGTTTGTCGTGTCACTCTTTGCAGTGATGATACTTGGCAACTTGCCCCCTCTTTCCATGATAGAGGGAGCATTTCTCAAATACTTTGGGATTCCTGTGGCTTTCACATGGTTTATGTC 5040
S. suis         ATGGTTTGTCGTGTCACTCTTTGCAGTGATGATACTTGGCAACTTGCCCCCTCTTTCCATGATAGAGGGAGCATTTCTCAAATACTTTGGGATTCCTGTGGCTTTCACATGGTTTATGCC 5039
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S. parauberis   TACAAAAACTTTTGATGGTAAAAAGCCTTATGGATTTTTGAAGTCTGTCATTGCTTATGCACTGCGACCAAAGCTGACCTATGCAGGAAAAAAAGTAACGCTTGGCAGAAACCAGCCACA 5159
S. agalatiae    TACAAAAACTTTTGATGGTAAAAAGCCTTATGGATTTTTGAAGTCTGTCATTGCTTATGCACTGCGACCAAAGCTGACCTATGCAGGAAAAAAAGTAACGCTTGGCAGAAACCAGCCACA 5159
pCF10           TACAAAAACTTTTGATGGTAAAAAGCCTTATGGATTTTTGAAGTCTGTCATTGCTTATGCACTGCGACCAAAGCTGACCTATGCAGGAAAAAAAGTAACGCTTGGCAGAAACCAGCCACA 5159
E. faecalis     TACAAAAACTTTTGATGGTAAAAAGCCTTATGGATTTTTGAAGTCTGTCATTGCTTATGCACTGCGACCAAAGCTGACCTATGCAGGAAAAAAAGTAACGCTTGGCAGAAACCAGCCACA 5160
S. suis         TACAAAAACTTTTGACGGTAAAAAGCCTTATGGATTTTTGAAGTCTGTCATTGCTTATGCACTGCGACCAAAGCTGACCTATGCAGGAAAAAAAGTAACGCTTGGCAGAAACCAGCCACA 5159
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S. parauberis   AGAAGCCATTACAGCAGTTAGGAGTGAATTTTATGGCATATCCAATTAAATACATTGAAAACAATCTCGTCTGGAATAAAGACGGGGAATGTTATGCTTACTATGAGCTTGTTCCTTACA 5279
S. agalatiae    AGAAGCCATTACAGCAGTTAGGAGTGAATTTTATGGCATATCCAATTAAATACATTGAAAACAATCTCGTCTGGAATAAAGACGGGGAATGTTATGCTTACTATGAGCTTGTTCCTTACA 5279
pCF10           AGAAGCCATTACAGCAGTTAGGAGTGAATTTTATGGCATATCCAATTAAATACATTGAAAACAATCTCGTCTGGAATAAAGACGGGGAATGTTATGCTTACTATGAGCTTGTTCCTTACA 5279
E. faecalis     AGAAGCCATTACAGCAGTTAGGAGTGAATTTTATGGCATATCCAATTAAATACATTGAAAACAATCTCGTCTGGAATAAAGACGGGGAATGTTATGCTTACTATGAGCTTGTTCCTTACA 5280
S. suis         AGAAGCCATTACAGCAGTTAGGAGTGAATTTTATGGCATATCCAATTAAATACATTGAAAACAATCTCGTCTGGAATAAAGACGGGGAATGTTATGCTTACTATGAGCTTGTTCCTTACA 5279
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S. parauberis   ATTACTCATTTCTAAGTCCAGAACAGAAAATACAAGTGCATGATTCTTTCAGACAGCTTATCGCACAAAATCGTGATGGCAAAATTCATGCTTTACAAATCAGTACAGAATCCAGCATAC 5399
S. agalatiae    ATTACTCATTTCTAAGTCCAGAACAGAAAATACAAGTGCATGATTCTTTCAGACAGCTTATCGCACAAAATCGTGATGGCAAAATTCATGCTTTACAAATCAGTACAGAATCCAGCATAC 5399
pCF10           ATTACTCATTTCTAAGTCCAGAACAGAAAATACAAGTGCATGATTCTTTCAGACAGCTTATCGCACAAAATCGTGATGGCAAAATTCATGCTTTACAAATCAGTACAGAATCCAGCATAC 5399
E. faecalis     ATTACTCATTTCTAAGTCCAGAACAGAAAATACAAGTGCATGATTCTTTCAGACAGCTTATCGCACAAAATCGTGATGGCAAAATTCATGCTTTACAAATCAGTACAGAATCCAGCATAC 5400
S. suis         ATTACTCATTTCTAAGTCCAGAACAGAAAATACAAGTGCATGATTCTTTCAGACAGCTTATCGCACAAAATCGTGATGGCAAAATTCATGCTTTACAAATCAGTACAGAATCCAGCATAC 5399
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S. parauberis   GTTCTGCACAAGAGCGTTCCAAAAATGAAGTCACTGGCAAGCTCAAAGCGGTTGCCTATGACAAAATCGACCAACAGACAGACGCTTTAATATCCATGATTGGCGAAAATCAAGTGAACT 5519
S. agalatiae    GTTCTGCACAAGAGCGTTCCAAAAATGAAGTCACTGGCAAGCTCAAAGCGGTTGCCTATGACAAAATCGACCAACAGACAGACGCTTTAATATCCATGATTGGCGAAAATCAAGTGAACT 5519
pCF10           GTTCTGCACAAGAGCGTTCCAAAAATGAAGTCACTGGCAAGCTCAAAGCGGTTGCCTATGACAAAATCGACCAACAGACAGACGCTTTAATATCCATGATTGGCGAAAATCAAGTGAACT 5519
E. faecalis     GTTCTGCACAAGAGCGTTCCAAAAATGAAGTCACTGGCAAGCTCAAAGCGGTTGCCTATGACAAAATCGACCAACAGACAGACGCTTTAATATCCATGATTGGCGAAAATCAAGTGAACT 5520
S. suis         GTTCTGCACAAGAGCGTTCCAAAAATGAAGTCACTGGCAAGCTCAAAGCGGTTGCCTATGACAAAATCGACCAACAGACAGACGCTTTAATATCCATGATTGGCGAAAATCAAGTGAACT 5519
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S. parauberis   ACCGTTTCTTTATCGGCTTTAAGTTGCTTCTCAACGATCAGGAGTTTTCTATGAAAAGTCTTACCGTTGAAGCAAAAAATGCTTTGTCTGATTTTGTCTATGATGTGAACCATAAGCTGA 5639
S. agalatiae    ACCGTTTCTTTATCGGCTTTAAGTTGCTTCTCAACGATCAGGAGTTTTCTATGAAAAGTCTTACCGTTGAAGCAAAAAATGCTTTGTCTGATTTTGTCTATGATGTGAACCATAAGCTGA 5639
pCF10           ACCGTTTCTTTATCGGCTTTAAGTTGCTTCTCAACGATCAGGAGTTTTCTATGAAAAGTCTTACCGTTGAAGCAAAAAATGCTTTGTCTGATTTTGTCTATGATGTGAACCATAAGCTGA 5639
E. faecalis     ACCGTTTCTTTATCGGCTTTAAGTTGCTTCTCAACGATCAGGAGTTTTCTATGAAAAGTCTTACCGTTGAAGCAAAAAATGCTTTGTCTGATTTTGTCTATGATGTGAACCATAAGCTGA 5640
S. suis         ACCGTTTCTTTATCGGCTTTAAGTTGCTTCTCAACGATCAGGAGTTTTCTATGAAAAGTCTTACCGTTGAAGCAAAAAATGCTTTGTCTGATTTTGTCTATGATGTGAACCATAAGCTGA 5639
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S. parauberis   TGGGCGATTTTGTTAGTATGAGTAATGATGAAATCCTGCGTTTTCAGAAGATGGAAAAGCTCTTAGAAAATAAAATCTCTCGTCGTTTCAAAATCCGCAGGTTAGATAAGGACGACTTCG 5759
S. agalatiae    TGGGCGATTTTGTTAGTATGAGTAATGATGAAATCCTGCGTTTTCAGAAGATGGAAAAGCTCTTAGAAAATAAAATCTCTCGTCGTTTCAAAATCCGCAGGTTAGATAAGGACGACTTCG 5759
pCF10           TGGGCGATTTTGTTAGTATGAGTAATGATGAAATCCTGCGTTTTCAGAAGATGGAAAAGCTCTTAGAAAATAAAATCTCTCGTCGTTTCAAAATCCGCAGGTTAGATAAGGACGACTTCG 5759
E. faecalis     TGGGCGATTTTGTTAGTATGAGTAATGATGAAATCCTGCGTTTTCAGAAGATGGAAAAGCTCTTAGAAAATAAAATCTCTCGTCGTTTCAAAATCCGCAGGTTAGATAAGGACGACTTCG 5760
S. suis         TGGGCGATTTTGTTAGTATGAGTAATGATGAAATCCTGCGTTTTCAGAAGATGGAAAAGCTCTTAGAAAATAAAATCTCTCGTCGTTTCAAAATCCGCAGGTTAGATAAGGACGACTTCG 5759
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S. parauberis   GCTATCTGATTGAACACCTTTACGGACAGACAGGCACTGCCTATGAAGAGTATGAGTACCATCTATCAAAGAAAAAGCTGGATAATGAAACGCTGATTAAATACTATGACTTGATTAAGC 5879
S. agalatiae    GCTATCTGATTGAACACCTTTACGGACAGACAGGCACTGCCTATGAAGAGTATGAGTACCATCTATCAAAGAAAAAGCTGGATAATGAAACGCTGATTAAATACTATGACTTGATTAAGC 5879
pCF10           GCTATCTGATTGAACACCTTTACGGACAGACAGGCACTGCCTATGAAGAGTATGAGTACCATCTATCAAAGAAAAAGCTGGATAATGAAACGCTGATTAAATACTATGACTTGATTAAGC 5879
E. faecalis     GCTATCTGATTGAACACCTTTACGGACAGACAGGCACTGCCTATGAAGAGTATGAGTACCATCTATCAAAGAAAAAGCTGGATAATGAAACGCTGATTAAATACTATGACTTGATTAAGC 5880
S. suis         GCTATCTGATTGAACACCTTTACGGACAGACAGGCACTGCCTATGAAGAGTATGAGTACCATCTATCAAAGAAAAAGCTGGATAATGAAACGCTGATTAAATACTATGACTTGATTAAGC 5879
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S. parauberis   CTACTCGCTGTTTGGTGGAAGAAAAACAGCGATATTTGAAAATCCAGCAGGAAGATGAAACCGTCTATGTAGCTTACTTTACCATTAACAGCATTGTCGGAGAACTGGACTTCCCGTCCT 5999
S. agalatiae    CTACTCGCTGTTTGGTGGAAGAAAAACAGCGATATTTGAAAATCCAGCAGGAAGATGAAACCGTCTATGTAGCTTACTTTACCATTAACAGCATTGTCGGAGAACTGGACTTCCCGTCCT 5999
pCF10           CTACTCGCTGTTTGGTGGAAGAAAAACAGCGATATTTGAAAATCCAGCAGGAAGATGAAACCGTCTATGTAGCTTACTTTACCATTAACAGCATTGTCGGAGAACTGGACTTCCCGTCCT 5999
E. faecalis     CTACTCGCTGTTTGGTGGAAGAAAAACAGCGATATTTGAAAATCCAGCAGGAAGATGAAACCGTCTATGTAGCTTACTTTACCATTAACAGCATTGTCGGAGAACTGGACTTCCCGTCCT 6000
S. suis         CTACTCGCTGTTTGGTGGAAGAAAAACAGCGATATTTGAAAATCCAGCAGGAAGATGAAACCGTCTATGTAGCTTACTTTACCATTAACAGCATTGTCGGAGAACTGGACTTCCCGTCCT 5999
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S. parauberis   CTGAAATCTTCTACTACCAGCAACAGCAATTTACATTCCCGATTGATACGTCAATGAATGTGGAAATTGTAGCGAATCGTAAAGCCCTATCTACTGTCCGCAATAAAAAGAAAGAACTGA 6119
S. agalatiae    CTGAAATCTTCTACTACCAGCAACAGCAATTTACATTCCCGATTGATACGTCAATGAATGTGGAAATTGTAGCGAATCGTAAAGCCCTATCTACTGTCCGCAA-AAAAAGAAAGAACTGA 6118
pCF10           CTGAAATCTTCTACTACCAGCAACAGCAATTTACATTCCCGATTGATACGTCAATGAATGTGGAAATTGTAGCGAATCGTAAAGCCCTATCTACTGTCCGCAATAAAAAGAAAGAACTGA 6119
E. faecalis     CTGAAATCTTCTACTACCAGCAACAGCAATTTACATTCCCGATTGATACGTCAATGAATGTGGAAATTGTAGCGAATCGTAAAGCCCTATCTACTGTCCGCAATAAAAAGAAAGAACTGA 6120
S. suis         CTGAAATCTTCTACTACCAGCAACAGCAATTTACATTCCCGATTGATACGTCAATGAATGTGGAAATTGTAGCGAATCGTAAAGCCCTATCTACTGTCCGCAATAAAAAGAAAGAACTGA 6119
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S. parauberis   AAGACTTGGATAACCACGCTTGGCAAAGTGATAATGAAACCAGCTCCAATGTGGCGGAAGCTCTGGAAAGTGTGAATGAGCTGGAAACCAATTTAGACCAAAGCAAGGAATCTATGTACA 6239
S. agalatiae    AAGACTTGGATAACCACGCTTGGCAAAGTGATAATGAAACCAGCTCCAATGTGGCGGAAGCTCTGGAAAGTGTGAATGAGCTGGAAACCAATTTAGACCAAAGCAAGGAATCTATGTACA 6238
pCF10           AAGACTTGGATAACCACGCTTGGCAAAGTGATAATGAAACCAGCTCCAATGTGGCGGAAGCTCTGGAAAGTGTGAATGAGCTAGAAACCAATTTAGACCAAAGCAAGGAATCTATGTACA 6239
E. faecalis     AAGACTTGGATAACCACGCTTGGCAAAGTGATAATGAAACCAGCTCCAATGTGGCGGAAGCTCTGGAAAGTGTGAATGAGCTGGAAACCAATTTAGACCAAAGCAAGGAATCTATGTACA 6240
S. suis         AAGACTTGGATAACCACGCTTGGCAAAGTGATAATGAAACCAGCTCCAATGTGGCGGAAGCTCTGGAAAGTGTGAATGAGCTGGAAACCAATTTAGACCAAAGCAAGGAATCTATGTACA 6239
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S. parauberis   AGCTGTCTTATGTGGTAAGGGTATCAGCAAATGATCTTGACGAACTCAAACGTCGTTGTAATGAAGTGAAAGATTTTTATGACGATTTAAGCGTAAAACTGGTACGACCATTTGGGGATA 6359
S. agalatiae    AGCTGTCTTATGTGGTAAGGGTATCAGCAAATGATCTTGACGAACTCAAACGTCGTTGTAATGAAGTGAAAGATTTTTATGACGATTTAAGCGTAAAACTGGTACGACCATTTGGGGATA 6358
pCF10           AGCTGTCTTATGTGGTAAGGGTATCAGCAAATGATCTTGACGAACTCAAACGTCGTTGTAATGAAGTGAAAGATTTTTATGACGATTTAAGCGTAAAACTGGTACGACCATTTGGGGATA 6359
E. faecalis     AGCTGTCTTATGTGGTAAGGGTATCAGCAAATGATCTTGACGAACTCAAACGTCGTTGTAATGAAGTGAAAGATTTTTATGACGATTTAAGCGTAAAACTGGTACGACCATTTGGGGATA 6360
S. suis         AGCTGTCTTATGTGGTAAGGGTATCAGCAAATGATCTTGACGAACTCAAACGTCGTTGTAATGAAGTGAAAGATTTTTATGACGATTTAAGCGTAAAACTGGTACGACCATTTGGGGATA 6359
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S. parauberis   TGCTCGGCTTACATGAAGAATTTTTACCTGCCAGCAAGCGTTATATGAATGATTATATTCAATACGTGACCTCTGATTTCCTCGCTGGTTTAGGTTTTGGTGCTACTCAAATGCTGGGGG 6479
S. agalatiae    TGCTCGGCTTACATGAAGAATTTTTACCTGCCAGCAAGCGTTATATGAATGATTATATTCAATACGTGACCTCTGATTTCCTCGCTGGTTTAGGTTTTGGTGCTACTCAAATGCTGGGGG 6478
pCF10           TGCTCGGCTTACATGAAGAATTTTTACCTGCCAGCAAGCGTTATATGAATGATTATATTCAATACGTGACCTCTGATTTCCTCGCTGGTTTAGGTTTTGGTGCTACTCAAATGCTGGGGG 6479
E. faecalis     TGCTCGGCTTACATGAAGAATTTTTACCTGCCAGCAAGCGTTATATGAATGATTATATTCAATACGTGACCTCTGATTTCCTCGCTGGTTTAGGTTTTGGTGCTACTCAAATGCTGGGGG 6480
S. suis         TGCTCGGCTTACATGAAGAATTTTTACCTGCCAGCAAGCGTTATATGAATGATTATATTCAATACGTGACCTCTGATTTCCTCGCTGGTTTAGGTTTTGGTGCTACTCAAATGCTGGGGG 6479
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S. parauberis   AAAATGAGGGGATTTATGTTGGCTACAGCTTAGATACTGGACGCAATGTCTATCTGAAACCTGCTCTTGCCAGTCAAGGGGTTAAGGGTTCAGTAACCAATGCGTTAGCGTCGGCTTTTG 6599
S. agalatiae    AAAATGAGGGGATTTATGTTGGCTACAGCTTAGATACTGGACGCAATGTCTATCTGAAACCTGCTCTTGCCAGTCAAGGGGTTAAGGGTTCAGTAACCAATGCGTTAGCGTCGGCTTTTG 6598
pCF10           AAAATGAGGGGATTTATGTTGGCTACAGCTTAGATACTGGACGCAATGTCTATCTGAAACCTGCTCTTGCCAGTCAAGGGGTTAAGGGTTCAGTAACCAATGCGTTAGCGTCGGCTTTTG 6599
E. faecalis     AAAATGAGGGGATTTATGTTGGCTACAGCTTAGATACTGGACGCAATGTCTATCTGAAACCTGCTCTTGCCAGTCAAGGGGTTAAGGGTTCAGTAACCAATGCGTTAGCGTCGGCTTTTG 6600
S. suis         AAAATGAGGGGATTTATGTTGGCTACAGCTTAGATACTGGACGCAATGTCTATCTGAAACCTGCTCTTGCCAGTCAAGGGGTTAAGGGTTCAGTAACCAATGCGTTAGCGTCGGCTTTTG 6599
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S. parauberis   TTGGTTCGCTGGGTGGTGGTAAATCCTTTGCGAATAACCTTATCGTCTATTATGCGGTGCTTTATGGGGCACAAGCAGTGATTGTAGACCCAAAAGCAGAACGTGGCAGATGGAAAGAAA 6719
S. agalatiae    TTGGTTCGCTGGGTGGTGGTAAATCCTTTGCGAATAACCTTATCGTCTATTATGCGGTGCTTTATGGGGCACAAGCAGTGATTGTAGACCCAAAAGCAGAACGTGGCAGATGGAAAGAAA 6718
pCF10           TTGGTTCGCTGGGTGGTGGTAAATCCTTTGCGAATAACCTTATCGTCTATTATGCGGTGCTTTATGGGGCACAAGCAGTGATTGTAGACCCAAAAGCAGAACGTGGCAGATGGAAAGAAA 6719
E. faecalis     TTGGTTCGCTGGGTGGTGGTAAATCCTTTGCGAATAACCTTATCGTCTATTATGCGGTGCTTTATGGGGCACAAGCAGTGATTGTAGACCCAAAAGCAGAACGTGGCAGATGGAAAGAAA 6720
S. suis         TTGGTTCGCTGGGTGGTGGTAAATCCTTTGCGAATAACCTTATCGTCTATTATGCGGTGCTTTATGGGGCACAAGCAGTGATTGTAGACCCAAAAGCAGAACGTGGCAGATGGAAAGAAA 6719
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S. parauberis   CCTTGCCAGAGATTTCCCATGAAATCAATATCGTCACTCTGACTTCTGATGAGAAAAACAAAGGCTTACTTGACCCTTATGTGATTATGAAAAATCCCAAAGATTCTGAATCACTGGCTA 6839
S. agalatiae    CCTTGCCAGAGATTTCCCATGAAATCAATATCGTCACTCTGACTTCTGATGAGAAAAACAAAGGCTTACTTGACCCTTATGTGATTATGAAAAATCCCAAAGATTCTGAATCACTGGCTA 6838
pCF10           CCTTGCCAGAGATTTCCCATGAAATCAATATCGTCACTCTGACTTCTGATGAGAAAAACAAAGGCTTACTTGACCCTTATGTGATTATGAAAAATCCCAAAGATTCTGAATCACTGGCTA 6839
E. faecalis     CCTTGCCAGAGATTTCCCATGAAATCAATATCGTCACTCTGACTTCTGATGAGAAAAACAAAGGCTTACTTGACCCTTATGTGATTATGAAAAATCCCAAAGATTCTGAATCACTGGCTA 6840
S. suis         CCTTGCCAGAGATTTCCCATGAAATCAATATCGTCACTCTGACTTCTGATGAGAAAAACAAAGGCTTACTTGACCCTTATGTGATTATGAAAAATCCCAAAGATTCTGAATCACTGGCTA 6839
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S. parauberis   TTGATATTCTGACATTCCTTACGGGGATTTCCTCTCGTGATGGGGAACGCTTCCCAATCCTTAGAAAAGCCATTCGTGCAGTAACCAATAGTGAAGTACGAGGGTTGATGAAAGTGATTG 6959
S. agalatiae    TTGATATTCTGACATTCCTTACGGGGATTTCCTCTCGTGATGGGGAACGCTTCCCAATCCTTAGAAAAGCCATTCGTGCAGTAACCAATAGTGAAGTACGAGGGTTGATGAAAGTGATTG 6958
pCF10           TTGATATTCTGACATTCCTTACGGGGATTTCCTCTCGTGATGGGGAACGCTTCCCAATCCTTAGAAAAGCCATTCGTGCAGTAACCAATAGTGAAGTACGAGGGTTGATGAAAGTGATTG 6959
E. faecalis     TTGATATTCTGACATTCCTTACGGGGATTTCCTCTCGTGATGGGGAACGCTTCCCAATCCTTAGAAAAGCCATTCGTGCAGTAACCAATAGTGAAGTACGAGGGTTGATGAAAGTGATTG 6960
S. suis         TTGATATTCTGACATTCCTTACGGGGATTTCCTCTCGTGATGGGGAACGCTTCCCAATCCTTAGAAAAGCCATTCGTGCAGTAACCAATAGTGAAGTACGAGGGTTGATGAAAGTGATTG 6959
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S. parauberis   AGGAATTACGGGTTGAGAATACGCCACTAAGTACCAGTATAGCCGACCATATCGAAAGTTTTACAGACTATGACTTTGCACATTTATTATTCAGTAATGGTTATGTGGAGCAGTCTATCA 7079
S. agalatiae    AGGAATTACGGGTTGAGAATACGCCACTAAGTACCAGTATAGCCGACCATATCGAAAGTTTTACAGACTATGACTTTGCACATTTATTATTCAGTAATGGTTATGTGGAGCAGTCTATCA 7078
pCF10           AGGAATTACGGGTTGAGAATACGCCACTAAGTACCAGTATAGCCGACCATATCGAAAGTTTTACAGACTATGACTTTGCACATTTATTATTCAGTAATGGTTATGTGGAGCAGTCTATCA 7079
E. faecalis     AGGAATTACGGGTTGAGAATACGCCACTAAGTACCAGTATAGCCGACCATATCGAAAGTTTTACAGACTATGACTTTGCACATTTATTATTCAGTAATGGTTATGTGGAGCAGTCTATCA 7080
S. suis         AGGAATTACGGGTTGAGAATACGCCACTAAGTACCAGTATAGCCGACCATATCGAAAGTTTTACAGACTATGACTTTGCACATTTATTATTCAGTAATGGTTATGTGGAGCAGTCTATCA 7079
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S. parauberis   GCTTAGAAAAACAACTGAACATTATACAGGTTGCGGACTTGGTACTTCCCGACAAGGAAACTTCCTTTGAGGAATATACCACTATGGAGCTTTTATCCGTTGCTATGCTGATTGTCATTA 7199
S. agalatiae    GCTTAGAAAAACAACTGAACATTATACAGGTTGCGGACTTGGTACTTCCCGACAAGGAAACTTCCTTTGAGGAATATACCACTATGGAGCTTTTATCCGTTGCTATGCTGATTGTCATTA 7198
pCF10           GCTTAGAAAAACAACTGAACATTATACAGGTTGCGGACTTGGTACTTCCCGACAAGGAAACTTCCTTTGAGGAATATACCACTATGGAGCTTTTATCCGTTGCTATGCTGATTGTCATTA 7199
E. faecalis     GCTTAGAAAAACAACTGAACATTATACAGGTTGCGGACTTGGTACTTCCCGACAAGGAAACTTCCTTTGAGGAATATACCACTATGGAGCTTTTATCCGTTGCTATGCTGATTGTCATTA 7200
S. suis         GCTTAGAAAAACAACTGAACATTATACAGGTTGCGGACTTGGTACTTCCCGACAAGGAAACTTCCTTTGAGGAATATACCACTATGGAGCTTTTATCCGTTGCTATGCTGATTGTCATTA 7199
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S. parauberis   GTACCTTTGCTTTAGACTTTATCCATACAGACCGAAGCATTTTCAAGATTGTAGATTTAGACGAAGCATGGAGCTTTTTACAGGTAGCACAAGGAAAAACACTATCTATGAAGCTGGTTC 7319
S. agalatiae    GTACCTTTGCTTTAGACTTTATCCATACAGACCGAAGCATTTTCAAGATTGTAGATTTAGACGAAGCATGGAGCTTTTTACAGGTAGCACAAGGAAAAACACTATCTATGAAGCTGGTTC 7318
pCF10           GTACCTTTGCTTTAGACTTTATCCATACAGACCGAAGCATTTTCAAGATTGTAGATTTAGACGAAGCATGGAGCTTTTTACAGGTAGCACAAGGAAAAACACTATCTATGAAGCTGGTTC 7319
E. faecalis     GTACCTTTGCTTTAGACTTTATCCATACAGACCGAAGCATTTTCAAGATTGTAGATTTAGACGAAGCATGGAGCTTTTTACAGGTAGCACAAGGAAAAACACTATCTATGAAGCTGGTTC 7320
S. suis         GTACCTTTGCTTTAGACTTTATCCATACAGACCGAAGCATTTTCAAGATTGTAGATTTAGACGAAGCATGGAGCTTTTTACAGGTAGCACAAGGAAAAACACTATCTATGAAGCTGGTTC 7319
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S. parauberis   GGGCTGGTCGTGCTATGAACGCTGGGGTATATTTCGTGACCCAAAATACAGACGACCTCTTAGATGAAAAACTGAAAAATAACCTCGGCTTAAAATTTGCATTTCGTTCCACTGACCTTA 7439
S. agalatiae    GGGCTGGTCGTGCTATGAACGCTGGGGTATATTTCGTGACCCAAAATACAGACGACCTCTTAGATGAAAAACTGAAAAATAACCTCGGCTTAAAATTTGCATTTCGTTCCACTGACCTTA 7438
pCF10           GGGCTGGTCGTGCTATGAACGCTGGGGTATATTTCGTGACCCAAAATACAGACGACCTCTTAGATGAAAAACTGAAAAATAACCTCGGCTTAAAATTTGCATTTCGTTCCACTGACCTTA 7439
E. faecalis     GGGCTGGTCGTGCTATGAACGCTGGGGTATATTTCGTGACCCAAAATACAGACGACCTCTTAGATGAAAAACTGAAAAATAACCTCGGCTTAAAATTTGCATTTCGTTCCACTGACCTTA 7440
S. suis         GGGCTGGTCGTGCTATGAACGCTGGGGTATATTTCGTGACCCAAAATACAGACGACCTCTTAGATGAAAAACTGAAAAATAACCTCGGCTTAAAATTTGCATTTCGTTCCACTGACCTTA 7439
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S. parauberis   ACGAGATTAAAAAGACCTTAGCCTTTTTTGGTGTAGACCCAGAGGACGAAAACAATCAGAAGCGATTGCGTGATTTGGAAAACGGGCAATGCCTTATCAGTGATTTATATGGTCGTGTCG 7559
S. agalatiae    ACGAGATTAAAAAGACCTTAGCCTTTTTTGGTGTAGACCCAGAGGACGAAAACAATCAGAAGCGATTGCGTGATTTGGAAAACGGGCAATGCCTTATCAGTGATTTATATGGTCGTGTCG 7558
pCF10           ACGAGATTAAAAAGACCTTAGCCTTTTTTGGTGTAGACCCAGAGGACGAAAACAATCAGAAGCGATTGCGTGATTTGGAAAACGGGCAATGCCTTATCAGTGATTTATATGGTCGTGTCG 7559
E. faecalis     ACGAGATTAAAAAGACCTTAGCCTTTTTTGGTGTAGACCCAGAGGACGAAAACAATCAGAAGCGATTGCGTGATTTGGAAAACGGGCAATGCCTTATCAGTGATTTATATGGTCGTGTCG 7560
S. suis         ACGAGATTAAAAAGACCCTAGCCTTTTTTGGTGTAGACCCAGAGGACGAAAACAATCAGAAGCGATTGCGTGATTTGGAAAACGGGCAATGCCTTATCAGTGATTTATATGGTCGTGTCG 7559
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S. parauberis   GTGTGATACAGTTCCACCCTGTATTTGAAGAACTGCTCCATGCCTTTGATACCAGACCACCTGTGCGAAAAGAGGTGTAAATGTGAAACCATCAATAGTAAACAGAATAAAATCAAACTG 7679
S. agalatiae    GTGTGATACAGTTCCACCCTGTATTTGAAGAACTGCTCCATGCCTTTGATACCAGACCACCTGTGCGAAAAGAGGTGTAAATGTGAAACCATCAATAGTAAACAGAATAAAATCAAACTG 7678
pCF10           GTGTGATACAGTTCCACCCTGTATTTGAAGAACTGCTCCATGCCTTTGATACCAGACCACCTGTGCGAAAAGAGGTGTAAATGTGAAACCATCAATAGTAAACAGAATAAAATCAAACTG 7679
E. faecalis     GTGTGATACAGTTCCACCCTGTATTTGAAGAACTGCTCCATGCCTTTGATACCAGACCACCTGTGCGAAAAGAGGTGTAAATGTGAAACCATCAATAGTAAACAGAATAAAATCAAACTG 7680
S. suis         GTGTGATACAGTTCCACCCTGTATTTGAAGAACTGCTCCATGCCTTTGATACCAGACCACCTGTGCGAAAAGAGGTGTAAATGTGAAACCATCAATAGTAAACAGAATAAAATCAAACTG 7679
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S. parauberis   GACGCTGAAACGTCTAGGTAAAGTGGCAATGACAGTGGCTTTCACACTTGTGATTGCCATTTTTCTTTTAGCCATGCTGGGAACGGTGGTTCAAGCTGCGGGCTTGGTAGATGATACGGT 7799
S. agalatiae    GACGCTGAAACGTCTAGGTAAAGTGGCAATGACAGTGGCTTTCACACTTGTGATTGCCATTTTTCTTTTAGCCATGCTGGGAACGGTGGTTCAAGCTGCGGGCTTGGTAGATGATACGGT 7798
pCF10           GACGCTGAAACGTCTAGGTAAAGTGGCAATGACAGTGGCTTTCACACTTGTGATTGCCATTTTTCTTTTAGCCATGCTGGGAACGGTGGTTCAAGCTGCGGGCTTGGTAGATGATACGGT 7799
E. faecalis     GACGCTGAAACGTCTAGGTAAAGTGGCAATGACAGTGGCTTTCACACTTGTGATTGCCATTTTTCTTTTAGCCATGCTGGGAACGGTGGTTCAAGCTGCGGGCTTGGTAGATGATACGGT 7800
S. suis         GACGCTGAAACGTCTAGGTAAAGTGGCAATGACAGTGGCTTTCACACTTGTGATTGCCATTTTTCTTTTAGCCATGCTGGGAACGGTGGTTCAAGCTGCGGGCTTGGTAGATGATACGGT 7799
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S. parauberis   CAATGTGGCAAATGAATACAGCCGATACCCACTTGAAAACTATCAACTGGATTTTTATGTGGATAATAGCTGGGGCTGGCTTCCGTGGAACTGGTCGGACGGGATTGGAAAACAGGTCAT 7919
S. agalatiae    CAATGTGGCAAATGAATACAGCCGATACCCACTTGAAAACTATCAACTGGATTTTTATGTGGATAATAGCTGGGGCTGGCTTCCGTGGAACTGGTCGGACGGGATTGGAAAACAGGTCAT 7918
pCF10           CAATGTGGCAAATGAATACAGCCGATACCCACTTGAAAACTATCAACTGGATTTTTATGTGGATAATAGCTGGGGCTGGCTTCCGTGGAACTGGTCGGACGGGATTGGAAAACAGGTCAT 7919
E. faecalis     CAATGTGGCAAATGAATACAGCCGATACCCACTTGAAAACTATCAACTGGATTTTTATGTGGATAATAGCTGGGGCTGGCTTCCGTGGAACTGGTCGGACGGGATTGGAAAACAGGTCAT 7920
S. suis         CAATGTGGCAAATGAATACAGCCGATACCCACTTGAAAACTATCAACTGGATTTTTATGTGGATAATAGCTGGGGCTGGCTTCCGTGGAACTGGTCGGACGGGATTGGAAAACAGGTCAT 7919
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S. parauberis   GTATGGACTATATGCCATTACCAATTTTATTTGGACAATCAGTTTGTATGTTTCCAATGCGACAGGTTACTTAGTACAGGAAGCCTATTCCTTAGACTTCATTTCCGCTACAGCAGATTC 8039
S. agalatiae    GTATGGACTATATGCCATTACCAATTTTATTTGGACAATCAGTTTGTATGTTTCCAATGCGACAGGTTACTTAGTACAGGAAGCCTATTCCTTAGACTTCATTTCCGCTACAGCAGATTC 8038
pCF10           GTATGGACTATATGCCATTACCAATTTTATTTGGACAATCAGTTTGTATGTTTCCAATGCGACAGGTTACTTAGTACAGGAAGCCTATTCCTTAGACTTCATTTCCGCTACAGCAGATTC 8039
E. faecalis     GTATGGACTATATGCCATTACCAATTTTATTTGGACAATCAGTTTGTATGTTTCCAATGCGACAGGTTACTTAGTACAGGAAGCCTATTCCTTAGACTTCATTTCCGCTACAGCAGATTC 8040
S. suis         GTATGGACTATATGCCATTACCAATTTTATTTGGACAATCAGTTTGTATGTTTCCAATGCGACAGGTTACTTAGTACAGGAAGCCTATTCCTTAGACTTCATTTCCGCTACAGCAGACTC 8039
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S. parauberis   CATTGGTAAGAATATGCAGACCTTAGCTGGTGTGAGTGCAAACGGATTTTCAACAGAGGGTTTCTATGTTGGATTCCTCTTACTCTTGATTTTGGTTCTTGGGGTTTATGTTGCCTATAC 8159
S. agalatiae    CATTGGTAAGAATATGCAGACCTTAGCTGGTGTGAGTGCAAACGGATTTTCAACAGAGGGTTTCTATGTTGGATTCCTCTTACTCTTGATTTTGGTTCTTGGGGTTTATGTTGCCTATAC 8158
pCF10           CATTGGTAAGAATATGCAGACCTTAGCTGGTGTGAGTGCAAACGGATTTTCAACAGAGGGTTTCTATGTTGGATTCCTCTTACTCTTGATTTTGGTTCTTGGGGTTTATGTTGCCTATAC 8159
E. faecalis     CATTGGTAAGAATATGCAGACCTTAGCTGGTGTGAGTGCAAACGGATTTTCAACAGAGGGTTTCTATGTTGGATTCCTCTTACTCTTGATTTTGGTTCTTGGGGTTTATGTTGCCTATAC 8160
S. suis         CATTGGTAAGAATATGCAGACCTTAGCTGGTGTGAGTGCAAACGGATTTTCAACAGAGGGTTTCTATGTTGGATTCCTCTTACTCTTGATTTTGGTTCTTGGGGTTTATGTTGCCTATAC 8159
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S. parauberis   GGGACTGATAAAGAGAGAAACCACAAAGGCAATTCATGCCATTATGAATTTTGTGCTGGTGTTTATCCTATCGGCTTCCTTTATTGCCTACGCTCCCGACTACATTAAAAAAATCAATGA 8279
S. agalatiae    GGGACTGATAAAGAGAGAAACCACAAAGGCAATTCATGCCATTATGAATTTTGTGCTGGTGTTTATCCTATCGGCTTCCTTTATTGCCTACGCTCCCGACTACATTAAAAAAATCAATGA 8278
pCF10           GGGACTGATAAAGAGAGAAACCACAAAGGCAATTCATGCCATTATGAATTTTGTGCTGGTGTTTATCCTATCGGCTTCCTTTATTGCCTACGCTCCCGACTACATTAAAAAAATCAATGA 8279
E. faecalis     GGGACTGATAAAGAGAGAAACCACAAAGGCAATTCATGCCATTATGAATTTTGTGCTGGTGTTTATCCTATCGGCTTCCTTTATTGCCTACGCTCCCGACTACATTAAAAAAATCAATGA 8280
S. suis         GGGACTGATAAAGAGAGAAACCACAAAGGCAATTCATGCCATTATGAATTTTGTGCTGGTGTTTATCCTATCGGCTTCCTTTATTGCCTACGCTCCCGACTACATTAAAAAAATCAATGA 8279
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S. parauberis   CTTTTCATCAGACATCAGTAATGCCAGTTTATCACTTGGCACGAAGATTGTCATGCCCCATTCCGATAGTCAAGGCAAGGACAGCGTGGACTTAATCAGAGATAGCCTGTTTTCCATACA 8399
S. agalatiae    CTTTTCATCAGACATCAGTAATGCCAGTTTATCACTTGGCACGAAGATTGTCATGCCCCATTCCGATAGTCAAGGCAAGGACAGCGTGGACTTAATCAGAGATAGCCTGTTTTCCATACA 8398
pCF10           CTTTTCATCAGACATCAGTAATGCCAGTTTATCACTTGGCACGAAGATTGTCATGCCCCATTCCGATAGTCAAGGCAAGGACAGCGTGGACTTAATCAGAGATAGCCTGTTTTCCATACA 8399
E. faecalis     CTTTTCATCAGACATCAGTAATGCCAGTTTATCACTTGGCACGAAGATTGTCATGCCCCATTCCGATAGTCAAGGCAAGGACAGCGTGGACTTAATCAGAGATAGCCTGTTTTCCATACA 8400
S. suis         CTTTTCATCAGACATCAGTAATGCCAGTTTATCACTTGGCACGAAGACTGTCATGCCCCATTCCGATAGTCAAGGCAAGGACAGCGTGGACTTAATCAGAGATAGCCTGTTTTCCATACA 8399
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S. parauberis   GGTTCAGCAACCGTGGCTACTGCTTCAATACAACAGTTCAGACATTGAAAGTATCGGTATTGACCGTGTGGAAAGCCTGCTCTCCACCAGCCCAGATTCCAACAATGGCGAAGACAGAGA 8519
S. agalatiae    GGTTCAGCAACCGTGGCTACTGCTTCAATACAACAGTTCAGACATTGAAAGTATCGGTATTGACCGTGTGGAAAGCCTGCTCTCCACCAGCCCAGATTCCAACAATGGCGAAGACAGAGA 8518
pCF10           GGTTCAGCAACCGTGGCTACTGCTTCAATACAACAGTTCAGACATTGAAAGTATCGGTATTGACCGTGTGGAAAGCCTGCTCTCCACCAGCCCAGATTCCAACAATGGCGAAGACAGAGA 8519
E. faecalis     GGTTCAGCAACCGTGGCTACTGCTTCAATACAACAGTTCAGACATTGAAAGTATCGGTATTGACCGTGTGGAAAGCCTGCTCTCCACCAGCCCAGATTCCAACAATGGCGAAGACAGAGA 8520
S. suis         GGTTCAGCAACCGTGGCTACTGCTTCAATACAACAGTTCAGACATTGAAAGTATCGGTATTGACCGTGTGGAAAGCCTGCTCTCCACCAGCCCAGATTCCAACAATGGCGAAGACAGAGA 8519
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S. parauberis   AAAAATTGTTGCGGAAGAAATTGAAGACAGAAGCAATACCAATCTAACCATTACAAAGACCATTAACCGTTTAGGTACAGTCTTCTTCCTATTTGTCTTCAATATTGGGATTTCCATATT 8639
S. agalatiae    AAAAATTGTTGCGGAAGAAATTGAAGACAGAAGCAATACCAATCTAACCATTACAAAGACCATTAACCGTTTAGGTACAGTCTTCTTCCTATTTGTCTTCAATATTGGGATTTCCATATT 8638
pCF10           AAAAATTGTTGCGGAAGAAATTGAAGACAGAAGCAATACCAATCTAACCATTACAAAGACCATTAACCGTTTAGGTACAGTCTTCTTCCTATTTGTCTTCAATATTGGGATTTCCATATT 8639
E. faecalis     AAAAATTGTTGCGGAAGAAATTGAAGACAGAAGCAATACCAATCTAACCATTACAAAGACCATTAACCGTTTAGGTACAGTCTTCTTCCTATTTGTCTTCAATATTGGGATTTCCATATT 8640
S. suis         AAAAATTGTTGCGGAAGAAATTGAAGACAGAAGCAATACCAATCTAACCATTACAAAGACCATTAACCGTTTAGGTACAGTCTTCTTCCTATTTGTCTTCAATATTGGGATTTCCATATT 8639
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S. parauberis   TGTATTCCTATTAACAGGAATCATGATTTTCTCGCAGGTACTTTTTATCATCTATGCTATGTTTCTGCCTGTGAGCTTTATTTTAAGCATGATTCCATCATTTGATGGTATGTCAAAACG 8759
S. agalatiae    TGTATTCCTATTAACAGGAATCATGATTTTCTCGCAGGTACTTTTTATCATCTATGCTATGTTTCTGCCTGTGAGCTTTATTTTAAGCATGATTCCATCATTTGATGGTATGTCAAAACG 8758
pCF10           TGTATTCCTATTAACAGGAATCATGATTTTCTCGCAGGTACTTTTTATCATCTATGCTATGTTTCTGCCTGTGAGCTTTATTTTAAGCATGATTCCATCATTTGATGGTATGTCAAAACG 8759
E. faecalis     TGTATTCCTATTAACAGGAATCATGATTTTCTCGCAGGTACTTTTTATCATCTATGCTATGTTTCTGCCTGTGAGCTTTATTTTAAGCATGATTCCATCATTTGATGGTATGTCAAAACG 8760
S. suis         TGTATTCCTATTAACAGGAATCATGATTTTCTCGCAGGTACTTTTTATCATCTATGCTATGTTTCTGCCTGTGAGCTTTATTTTAAGCATGATTCCATCATTTGATGGTATGTCAAAACG 8759
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S. parauberis   AGCCATAACAAAGCTCTTTAATACCATTTTGACACGAGCTGGAATCACATTGATTATTACGACAGCATTTAGTATTTCAACCATGCTCTATACCTTATCGGCTGGTTATCCGTTCTTTTT 8879
S. agalatiae    AGCCATAACAAAGCTCTTTAATACCATTTTGACACGAGCTGGAATCACATTGATTATTACGACAGCATTTAGTATTTCAACCATGCTCTATACCTTATCGGCTGGTTATCCGTTCTTTTT 8878
pCF10           AGCCATAACAAAGCTCTTTAATACCATTTTGACACGAGCTGGAATCACATTGATTATTACGACAGCATTTAGTATTTCAACCATGCTCTATACCTTATCGGCTGGTTATCCGTTCTTTTT 8879
E. faecalis     AGCCATAACAAAGCTCTTTAATACCATTTTGACACGAGCTGGAATCACATTGATTATTACGACAGCATTTAGTATTTCAACCATGCTCTATACCTTATCGGCTGGTTATCCGTTCTTTTT 8880
S. suis         AGCCATAACAAAGCTCTTTAATACCATTTTGACACGAGCTGGAATCACATTGATTATTACGACAGCATTTAGTATTTCAACCATGCTCTATACCTTATCGGCTGGTTATCCGTTCTTTTT 8879
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S. parauberis   GATTGCTTTTCTACAGATTGTGACCTTTGCAGGAATCTACTTCAAGCTGGGCGATTTAATGAGTATGTTTTCTCTACAGAGTAACGATTCTCAAAGTGTGGGAAGTCGTGTGATGAGAAA 8999
S. agalatiae    GATTGCTTTTCTACAGATTGTGACCTTTGCAGGAATCTACTTCAAGCTGGGCGATTTAATGAGTATGTTTTCTCTACAGAGTAACGATTCTCAAAGTGTGGGAAGTCGTGTGATGAGAAA 8998
pCF10           GATTGCTTTTCTACAGATTGTGACCTTTGCAGGAATCTACTTCAAGCTGGGCGATTTAATGAGTATGTTTTCTCTACAGAGTAACGATTCTCAAAGTGTGGGAAGTCGTGTGATGAGAAA 8999
E. faecalis     GATTGCTTTTCTACAGATTGTGACCTTTGCAGGAATCTACTTCAAGCTGGGCGATTTAATGAGTATGTTTTCTCTACAGAGTAACGATTCTCAAAGTGTGGGAAGTCGTGTGATGAGAAA 9000
S. suis         GATTGCTTTTCTACAGATTGTGACCTTTGCAGGAATCTACTTCAAGCTGGGCGATTTAATGAGTATGTTTTCTCTACAGAGTAACGATTCTCAAAGTGTGGGAAGTCGTGTGATGAGAAA 8999
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S. parauberis   ACCTCGTATGCTTATGCACGCTCACATGCACCGTCTACAGCGGAAACTTGGACGTTCCATGACTACTCTAGGGGCTGGGTCTGCCATTGTTACAGGTAAAAAAGGACAGTCGGGTTCGGG 9119
S. agalatiae    ACCTCGTATGCTTATGCACGCTCACATGCACCGTCTACAGCGGAAACTTGGACGTTCCATGACTACTCTAGGGGCTGGGTCTGCCATTGTTACAGGTAAAAAAGGACAGTCGGGTTCGGG 9118
pCF10           ACCTCGTATGCTTATGCACGCTCACATGCACCGTCTACAGCGGAAACTTGGACGTTCCATGACTACTCTAGGGGCTGGGTCTGCCATTGTTACAGGTAAAAAAGGACAGTCGGGTTCGGG 9119
E. faecalis     ACCTCGTATGCTTATGCACGCTCACATGCACCGTCTACAGCGGAAACTTGGACGTTCCATGACTACTCTAGGGGCTGGGTCTGCCATTGTTACAGGTAAAAAAGGACAGTCGGGTTCGGG 9120
S. suis         ACCTCGTATGCTTATGCACGCTCACATGCACCGTCTACAGCGGAAACTTGGACGTTCCATGACTACTCTAGGGGCTGGGTCTGCCATTGTTACAGGTAAAAAAGGACAGTCGGGTTCGGG 9119
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S. parauberis   GAGTTCTGCAAGGACACAAGCAGATCACTCCCGACCAGACGGAAAGGAAAAATCAACACTTGGAAAACGTATCGGTCAAACCATCGGTACAGTAGCTGATACCAAAGACAGAATGGTAGA 9239
S. agalatiae    GAGTTCTGCAAGGACACAAGCAGATCACTCCCGACCAGACGGAAAGGAAAAATCAACACTTGGAAAACGTATCGGTCAAACCATCGGTACAGTAGCTGATACCAAAGACAGAATGGTAGA 9238
pCF10           GAGTTCTGCAAGGACACAAGCAGATCACTCCCGACCAGACGGAAAGGAAAAATCAACACTTGGAAAACGTATCGGTCAAACCATCGGTACAGTAGCTGATACCAAAGACAGAATGGTAGA 9239
E. faecalis     GAGTTCTGCAAGGACACAAGCAGATCACTCCCGACCAGACGGAAAGGAAAAATCAACACTTGGAAAACGTATCGGTCAAACCATCGGTACAGTAGCTGATACCAAAGACAGAATGGTAGA 9240
S. suis         GAGTTCTGCAAGGACACAAGCAGATCACTCCCGACCAGACGGAAAGGAAAAATCAACACTTGGAAAACGTATCGGTCAAACCATCGGTACAGTAGCTGATACCAAAGACAGAATGGTAGA 9239
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S. parauberis    CACTGCTAGTGGTTTGAAAGAACAGGTTAAAGATTTGCCGACCAATGCAAGATATGCAGTATATCAAGGAAAATCCAAAGTAAAAGAGAATGTCCGTGATTTAACCAGTAGTATTTCTCA 9359
S. agalatiae     CACTGCTAGTGGTTTGAAAGAACAGGTTAAAGATTTGCCGACCAATGCAAGATATGCAGTATATCAAGGAAAATCCAAAGTAAAAGAGAATGTCCGTGATTTAACCAGTAGTATTTCTCA 9358
pCF10            CACTGCTAGTGGTTTGAAAGAACAGGTTAAAGATTTGCCGACCAATGCAAGATATGCAGTATATCAAGGAAAATCCAAAGTAAAAGAGAATGTCCGTGATTTAACCAGTAGTATTTCTCA 9359
E. faecalis      CACTGCTAGTGGTTTGAAAGAACAGGTTAAAGATTTGCCGACCAATGCAAGATATGCAGTATATCAAGGAAAATCCAAAGTAAAAGAGAATGTCCGTGATTTAACCAGTAGTATTTCTCA 9360
S. suis          CACTGCTAGTGGTTTGAAAGAACAGGTTAAAGATTTGCCGACCAATGCAAGATATGCAGTATATCAAGGAAAATCCAAAGTAAAAGAGAATGTCCGTGATTTAACCAGTAGTATTTCTCA 9359
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S. parauberis    AACCAAAGCGGACAGAGCCAGTGGACGCAAGGAACAGCAGGAACAAAGGCGAAAAACCATTGCGAAGCGTCGCTCTGAAATGGAACAGGTCAAACAGAAAAAACAGCCTGCTTCTTCTGT 9479
S. agalatiae     AACCAAAGCGGACAGAGCCAGTGGACGCAAGGAACAGCAGGAACAAAGGCGAAAAACCATTGCGAAGCGTCGCTCTGAAATGGAACAGGTCAAACAGAAAAAACAGCCTGCTTCTTCTGT 9478
pCF10            AACCAAAGCGGACAGAGCCAGTGGACGCAAGGAACAGCAGGAACAAAGGCGAAAAACCATTGCGAAGCGTCGCTCTGAAATGGAACAGGTCAAACAGAAAAAACAGCCTGCTTCTTCTGT 9479
E. faecalis      AACCAAAGCGGACAGAGCCAGTGGACGCAAGGAACAGCAGGAACAAAGGCGAAAAACCATTGCGAAGCGTCGCTCTGAAATGGAACAGGTCAAACAGAAAAAACAGCCTGCTTCTTCTGT 9480
S. suis          AACCAAAGCGGACAGAGCCAGTGGACGCAAGGAACAGCAGGAACAAAGGCGAAAAACCATTGCGAAGCGTCGCTCTGAAATGGAACAGGTCAAACAGAAAAAACAGCCTGCTTCTTCTGT 9479
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S. parauberis    TCATGAAAGACCGACTACAAGACAAGAACAATATCATGATGAACAGACCTCAAAACAGTCTAATATTCAGACTTCATATAAGGAATCTCAACAAGCCAAACAAGAGCGTCCAGCAGTTAA 9599
S. agalatiae     TCATGAAAGACCGACTACAAGACAAGAACAATATCATGATGAACAGACCTCAAAACAGTCTAATATTCAGACTTCATATAAGGAATCTCAACAAGCCAAACAAGAGCGTCCAGCAGTTAA 9598
pCF10            TCATGAAAGACCGACTACAAGACAAGAACAATATCATGATGAACAGACCTCAAAACAGTCTAATATTCAGACTTCATATAAGGAATCTCAACAAGCCAAACAAGAGCGTCCAGCAGTTAA 9599
E. faecalis      TCATGAAAGACCGACTACAAGACAAGAACAATATCATGATGAACAGACCTCAAAACAGTCTAATATTCAGACTTCATATAAGGAATCTCAACAAGCCAAACAAGAGCGTCCAGCAGTTAA 9600
S. suis          TCATGAAAGACCGACTACAAGACAAGAACAATATCATGATGAACAGACCTCAAAACAGTCTAATATTCAGACTTCATATAAGGAATCTCAACAAGCCAAACAAGAGCGTCCAGCAGTTAA 9599
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S. parauberis    GTCCGATTTTTCAAGTCCAAAAGTGGAACGC-AAGGCAATACCGTTCAAGAAAAAACCGTTCAAAAGCCAGCAACTTCAACCACTACAGCAGATAGAACTTCACAACGTCCAATCACAAA 9718
S. agalatiae     GTCCGATTTTTCAAGTCCAAAAGTGGAACGCCAAGGCAATACCGTTCAAGAAAAAACCGTTCAAAAGCCAGCAACTTCAACCACTACAGCAGATAGAACTTCACAACGTCCAATCACAAA 9718
pCF10            GTCCGATTTTTCAAGTCCAAAAGTGGAACGCCAAGGCAATACCGTTCAAGAAAAAACCGTTCAAAAGCCAGCAACTTCAACCACTACAGCAGATAGAACTTCACAACGTCCAATCACAAA 9719
E. faecalis      GTCCGATTTTTCAAGTCCAAAAGTGGAACGC-AAGGCAATACCGTTCAAGAAAAAACCGTTCAAAAGCCAGCAACTTCAACCACTACAGCAGATAGAACTTCACAACGTCCAATCACAAA 9719
S. suis          GTCCGATTTTTCAAGTCCAAAAGTGGAACGCCAAGGCAATACCGTTCAAGAAAAAACCGTTCAAAAGCCAGCAACTTCAACCACTACAGCAGATAGAACTTCACAACGTCCAATCACAAA 9719
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S. parauberis    AGAACGTCCGTCTACTGTTCAAAGAGTACCACTACAAAATACAAGAAGTAGACCACCAATCAAAACCGCCACCATTAAGAAAGTCGGTAAGAAACCATGAAGTTGAAAACTTTAGTGATT 9838
S. agalatiae     AGAACGTCCGTCTACTGTTCAAAGAGTACCACTACAAAATACAAGAAGTAGACCACCAATCAAAACCGCCACCATTAAGAAAGTCGGTAAGAAACCATGAAGTTGAAAACTTTAGTGATT 9838
pCF10            AGAACGTCCGTCTACTGTTCAAAGAGTACCACTACAAAATACAAGAAGTAGACCACCAATCAAAACCGCCACCATTAAGAAAGTCGGTAAGAAACCATGAAGTTGAAAACTTTAGTGATT 9839
E. faecalis      AGAACGTCCGTCTACTGTTCAAAGAGTACCACTACAAAATACAAGAAGTAGACCACCAATCAAAACCGCCACCATTAAGAAAGTCGGTAAGAAACCATGAAGTTGAAAACTTTAGTGATT 9839
S. suis          AGAACGTCCGTCTACTGTTCAAAGAGTACCACTACAAAATACAAGAAGTAGACCACCAATCAAAACCGCCACCATTAAGAAAGTCGGTAAGAAACCATGAAGTTGAAAACTTTAGTGATT 9839
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S. parauberis    GGTGGTTCTGGATTATTCTTGATGGTCTTCTCACTGCTTCTGTTTGTTGCCATTTTATTTTCAGATGAACAGGACAGCGGAATTTCCAATATTCATTATGGAGGTGTGAATGTTTCCGCA 9958
S. agalatiae     GGTGGTTCTGGATTATTCTTGATGGTCTTCTCACTGCTTCTGTTTGTTGCCATTTTATTTTCAGATGAACAGGACAGCGGAATTTCCAATATTCATTATGGAGGTGTGAATGTTTCCGCA 9958
pCF10            GGTGGTTCTGGATTATTCTTGATGGTCTTCTCACTGCTTCTGTTTGTTGCCATTTTATTTTCAGATGAACAGGACAGCGGAATTTCCAATATTCATTATGGAGGTGTGAATGTTTCCGCA 9959
E. faecalis      GGTGGTTCTGGATTATTCTTGATGGTCTTCTCACTGCTTCTGTTTGTTGCCATTTTATTTTCAGATGAACAGGACAGCGGAATTTCCAATATTCATTATGGAGGTGTGAATGTTTCCGCA 9959
S. suis          GGTGGTTCTGGGCTATTCTTGATGGTCTTCTCACTGCTTCTGTTTGTTGCCATTTTATTTTCAGATGAACAGGACAGCGGAATTTCCAATATTCATTATGGAGGTGTGAATGTTTCCGCA 9959
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S. parauberis    GAAGTGCTGGCTCATAAGCCTATGGTAGAAAAATATGCCAAAGAATATGGCGTTGAAGAATATGTCACCATACTTCTTGCGATTATACAGGTGGAATCGGGCGGTACTGCGGAAGATGTT 10078
S. agalatiae     GAAGTGCTGGCTCATAAGCCTATGGTAGAAAAATATGCCAAAGAATATGGCGTTGAAGAATATGTCAACATACTTCTTGCGATTATACAGGTGGAATCGGGCGGTACTGCGGAAGATGTT 10078
pCF10            GAAGTGCTGGCTCATAAGCCTATGGTAGAAAAATATGCCAAAGAATATGGCGTTGAAGAATATGTCAACATACTTCTTGCGATTATACAGGTGGAATCGGGCGGTACTGCGGAAGATGTT 10079
E. faecalis      GAAGTGCTGGCTCATAAGCCTATGGTAGAAAAATATGCCAAAGAATATGGCGTTGAAGAATATGTCAACATACTTCTTGCGATTATACAGGTGGAATCGGGCGGTACTGCGGAAGATGTT 10079
S. suis          GAAGTGCTGGCTCATAAGCCTATGGTAGAAAAATATGCCAAAGAATATGGCGTTGAAGAATATGTCAACATACTTCTTGCGATTATACAGGTGGAATCGGGCGGTACTGCGGAAGATGTT 10079
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S. parauberis    ATGCAGTCCTCGGAATCCCTCGGTCTTCCACCTAATTCATTGAGTACAGAAGAATCCATTAAGCAAGGTGTGAAGTATTTCAGTGAATTATTAGCCAGTAGCGAAAGGCTCAGTGTAGAT 10198
S. agalatiae     ATGCAGTCCTCGGAATCCCTCGGTCTTCCACCTAATTCATTGAGTACAGAAGAATCCATTAAGCAAGGTGTGAAGTATTTCAGTGAATTATTAGCCAGTAGCGAAAGGCTCAGTGTAGAT 10198
pCF10            ATGCAGTCCTCGGAATCCCTCGGTCTTCCACCTAATTCATTGAGTACAGAAGAATCCATTAAGCAAGGTGTGAAGTATTTCAGTGAATTATTAGCCAGTAGCGAAAGGCTCAGTGTAGAT 10199
E. faecalis      ATGCAGTCCTCGGAATCCCTCGGTCTTCCACCTAATTCATTGAGTACAGAAGAATCCATTAAGCAAGGTGTGAAGTATTTCAGTGAATTATTAGCCAGTAGCGAAAGGCTCAGTGTAGAT 10199
S. suis          ATGCAGTCCTCGGAATCCCTCGGTCTTCCACCTAATTCATTGAGTACAGAAGAATCCATTAAGCAAGGTGTGAAGTATTTCAGTGAATTATTAGCCAGTAGCGAAAGGCTCAGTGTAGAT 10199
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S. parauberis    TTAGAATCGGTTATCCAGTCCTACAATTATGGTGGTGGTTTCTTAGGGTATGTGGCTAATCGTGGAAATAAATATACCTTTGAACTGGCTCAAAGTTTCTCAAAAGAGTATTCAGGTGGC 10318
S. agalatiae     TTAGAATCGGTTATCCAGTCCTACAATTATGGTGGTGGTTTCTTAGGGTATGTGGCTAATCGTGGAAATAAATATACCTTTGAACTGGCTCAAAGTTTCTCAAAAGAGTATTCAGGTGGC 10318
pCF10            TTAGAATCGGTTATCCAGTCCTACAATTATGGTGGTGGTTTCTTAGGGTATGTGGCTAATCGTGGAAATAAATATACCTTTGAACTGGCTCAAAGTTTCTCAAAAGAGTATTCAGGTGGC 10319
E. faecalis      TTAGAATCGGTTATCCAGTCCTACAATTATGGTGGTGGTTTCTTAGGGTATGTGGCTAATCGTGGAAATAAATATACCTTTGAACTGGCTCAAAGTTTCTCAAAAGAGTATTCAGGTGGC 10319
S. suis          TTAGAATCGGTTATCCAGTCCTACAATTATGGTGGTGGTTTCTTAGGGTATGTGGCTAATCGTGGAAATAAATATACCTTTGAACTGGCTCAAAGTTTCTCAAAAGAGTATTCAGGTGGC 10319
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S. parauberis    GAAAAAGTGTCTTACCCCAATCCCATAGCCATACCTATCAATGGGGGCTGGCGATACAACTATGGCAATATGTTTTATGTGCAACTGGTAACGCAGTATCTTGTCACAACAGAGTTTGAT 10438
S. agalatiae     GAAAAAGTGTCTTACCCCAATCCCATAGCCATACCTATCAATGGGGGCTGGCGATACAACTATGGCAATATGTTTTATGTGCAACTGGTAACGCAGTATCTTGTCACAACAGAGTTTGAT 10438
pCF10            GAAAAAGTGTCTTACCCCAATCCCATAGCCATACCTATCAATGGGGGCTGGCGATACAACTATGGCAATATGTTTTATGTGCAACTGGTAACGCAGTATCTTGTCACAACAGAGTTTGAT 10439
E. faecalis      GAAAAAGTGTCTTACCCCAATCCCATAGCCATACCTATCAATGGGGGCTGGCGATACAACTATGGCAATATGTTTTATGTGCAACTGGTAACGCAGTATCTTGTCACAACAGAGTTTGAT 10439
S. suis          GAAAAAGTGTCTTACCCCAATCCCATAGCCATACCTATCAATGGGGGCTGGCGATACAACTATGGCAATATGTTTTATGTGCAACTGGTAACGCAGTATCTTGTCACAACAGAGTTTGAT 10439
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S. parauberis    GATGATACGGTACAAGCCATCATGGACGAAGCACTGAAATATGAGGGCTGGCGATACGTTTACGGTGGAGCTTCCCCGACTACTTCTTTTGATTGTAGCGGACTGACACAATGGACGTAT 10558
S. agalatiae     GATGATACGGTACAAGCCATCATGGACGAAGCACTGAAATATGAGGGCTGGCGATACGTTTACGGTGGAGCTTCCCCGACTACTTCTTTTGATTGTAGCGGACTGACACAATGGACGTAT 10558
pCF10            GATGATACGGTACAAGCCATCATGGACGAAGCACTGAAATATGAGGGCTGGCGATACGTTTACGGTGGAGCTTCCCCGACTACTTCTTTTGATTGTAGCGGACTGACACAATGGACGTAT 10559
E. faecalis      GATGATACGGTACAAGCCATCATGGACGAAGCACTGAAATATGAGGGCTGGCGATACGTTTACGGTGGAGCTTCCCCGACTACTTCTTTTGATTGTAGCGGACTGACACAATGGACGTAT 10559
S. suis          GATGATACGGTACAAGCCATCATGGACGAAGCACTGAAATATGAGGGCTGGCGATACGTTTACGGTGGAGCTTCCCCGACTACTTCTTTTGATTGTAGCGGACTGACACAATGGACGTAT 10559
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S. parauberis   GGAAAAGCTGGAATTAACTTACCACGAACCGCACAACAGCAATATGATGTGACCCAGCATATCCCACTATCGGAAGCACAAGCTGGCGATTTGGTTTTCTTTCATTCTACCTATAACGCT 10678
S. agalatiae    GGAAAAGCTGGAATTAACTTACCACGAACCGCACAACAGCAATATGATGTGACCCAGCATATCCCACTATCGGAAGCACAAGCTGGCGATTTGGTTTTCTTTCATTCTACCTATAACGCT 10678
pCF10           GGAAAAGCTGGAATTAACTTACCACGAACCGCACAACAGCAATATGATGTGACCCAGCATATCCCACTATCGGAAGCACAAGCTGGCGATTTGGTTTTCTTTCATTCTACCTATAACGCT 10679
E. faecalis     GGAAAAGCTGGAATTAACTTACCACGAACCGCACAACAGCAATATGATGTGACCCAGCATATCCCACTATCGGAAGCACAAGCTGGCGATTTGGTTTTCTTTCATTCTACCTATAACGCT 10679
S. suis         GGAAAAGCTGGAATTAACTTACCACGAACCGCACAACAGCAATATGATGTGACCCAGCATATCCCACTATCGGAAGCACAAGCTGGCGATTTGGTTTTCTTTCATTCTACCTATAACGCT 10679
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S. parauberis   GGCTCTTATATTACTCATGTTGGGATATACCTTGGCAATAACCGTATGTTTCATGCAGGCGACCCAATCGGTTATGCCGACTTAACAAGCCCCTACTGGCAACAGCATTTAGTGGGAGCA 10798
S. agalatiae    GGCTCTTATATTACTCATGTTGGGATATACCTTGGCAATAACCGTATGTTTCATGCAGGCGACCCAATCGGTTATGCCGACTTAACAAGCCCCTACTGGCAACAGCATTTAGTGGGAGCA 10798
pCF10           GGCTCTTATATTACTCATGTTGGGATATACCTTGGCAATAACCGTATGTTTCATGCAGGCGACCCAATCGGTTATGCCGACTTAACAAGCCCCTACTGGCAACAGCATTTAGTGGGAGCA 10799
E. faecalis     GGCTCTTATATTACTCATGTTGGGATATACCTTGGCAATAACCGTATGTTTCATGCAGGCGACCCAATCGGTTATGCCGACTTAACAAGCCCCTACTGGCAACAGCATTTAGTGGGAGCA 10799
S. suis         GGCTCTTATATTACTCATGTTGGGATATACCTTGGCAATAACCGTATGTTTCATGCAGGCGACCCAATCGGTTATGCCGACTTAACAAGCCCCTACTGGCAACAGCATTTAGTGGGAGCA 10799
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S. parauberis   GGACGAATCAAACAATGAGAAAGGAAGATTTAATGATGAAATTTAGAAAAAATCAGAATAAAGAAAAACAGATACCAAAGGAAAAGAAACCTCGTGTCTATAAGGTCAATCCTCATAAAA 10918
S. agalatiae    GGACGAATCAAACAATGAGAAAGGAAGATTTAATGATGAAATTTAGAAAAAATCAGAATAAAGAAAAACAGATACCAAAGGAAAAGAAACCTCGTGTCTATAAGGTCAATCCTCATAAAA 10918
pCF10           GGACGAATCAAACAATGAGAAAGGAAGATTTAATGATGAAATTTAGAAAAAATCAGAATAAAGAAAAACAGATACCAAAGGAAAAGAAACCTCGTGTCTATAAGGTCAATCCTCATAAAA 10919
E. faecalis     GGACGAATCAAACAATGAGAAAGGAAGATTTAATGATGAAATTTAGAAAAAATCAGAATAAAGAAAAACAGATACCAAAGGAAAAGAAACCTCGTGTCTATAAGGTCAATCCTCATAAAA 10919
S. suis         GGACGAATCAAACAATGAGAAAGGAAGATTTAATGATGAAATTTAGAAAAAATCAGAATAAAGAAAAACAGATACCAAAGGAAAAGAAACCTCGTGTCTATAAGGTCAATCCTCATAAAA 10919
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S. parauberis   AGGTTGTGATTGCCTTGTGGGTACTTTTAGGGCTTAGTTTCAGCTTTGCGATATTCAAGCACTTTACAGCTATAGATACTCATACTATTCACGAAACAACTATCATAGAAAAGGAATACG 11038
S. agalatiae    AGGTTGTGATTGCCTTGTGGGTACTTTTAGGGCTTAGTTTCAGCTTTGCGATATTCAAGCACTTTACAGCTATAGATACTCATACTATTCACGAAACAACTATCATAGAAAAGGAATACG 11038
pCF10           AGGTTGTGATTGCCTTGTGGGTACTTTTAGGGCTTAGTTTCAGCTTTGCGATATTCAAGCACTTTACAGCTATAGATACTCATACTATTCACGAAACAACTATCATAGAAAAGGAATACG 11039
E. faecalis     AGGTTGTGATTGCCTTGTGGGTACTTTTAGGGCTTAGTTTCAGCTTTGCGATATTCAAGCACTTTACAGCTATAGATACTCATACTATTCACGAAACAACTATCATAGAAAAGGAATACG 11039
S. suis         AGGTTGTGATTGCCTTGTGGGTACTTTTAGGGCTTAGTTTCAGCTTTGCGATATTCAAGCACTTTACAGCTATAGATACTCATACTATTCACGAAACAACTATCATAGAAAAGGAATACG 11039
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S. parauberis   TTGATACTCATCATGTAGAAAATTTTGTAGAGAACTTTGCGAAAGTCTACTATTCATGGGAGCAATCCGATAAGTCCATTGATAATCGAATGGAAAGTCTAAAAGGCTATCTGACAGATG 11158
S. agalatiae    TTGATACTCATCATGTAGAAAATTTTGTAGAGAACTTTGCGAAAGTCTACTATTCATGGGAGCAATCCGATAAGTCCATTGATAATCGAATGGAAAGTCTAAAAGGCTATCTGACAGATG 11158
pCF10           TTGATACTCATCATGTAGAAAATTTTGTAGAGAACTTTGCGAAAGTCTACTATTCATGGGAGCAATCCGATAAGTCCATTGATAATCGAATGGAAAGTCTAAAAGGCTATCTGACAGATG 11159
E. faecalis     TTGATACTCATCATGTAGAAAATTTTGTAGAGAACTTTGCGAAAGTCTACTATTCATGGGAGCAATCCGATAAGTCCATTGATAATCGAATGGAAAGTCTAAAAGGCTATCTGACAGATG 11159
S. suis         TTGATACTCATCATGTAGAAAATTTTGTAGAGAACTTTGCGAAAGTCTACTATTCATGGGAGCAATCCGATAAGTCCATTGATAATCGAATGGAAAGTCTAAAAGGCTATCTGACAGATG 11159
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S. parauberis   AACTTCAAGCTCTCAATGTTGATACAGTACGCAAAGATATTCCTGTATCGTCTTCTGTAAGAGGATTTCAGATATGGACGGTAGAGCCAACTGGCGACAATGAGTTTAATGTAACCTACA 11278
S. agalatiae    AACTTCAAGCTCTCAATGTTGATACAGTACGCAAAGATATTCCTGTATCGTCTTCTGTAAGAGGATTTCAGATATGGACGGTAGAGCCAACTGGCGACAATGAGTTTAATGTAACCTACA 11278
pCF10           AACTTCAAGCTCTCAATGTTGATACAGTACGCAAAGATATTCCTGTATCGTCTTCTGTAAGAGGATTTCAGATATGGACGGTAGAGCCAACTGGCGACAATGAGTTTAATGTAACCTACA 11279
E. faecalis     AACTTCAAGCTCTCAATGTTGATACAGTACGCAAAGATATTCCTGTATCGTCTTCTGTAAGAGGATTTCAGATATGGACGGTAGAGCCAACTGGCGACAATGAGTTTAATGTAACCTACA 11279
S. suis         AACTTCAAGCTCTCAATGTTGATACAGTACGCAAAGATATTCCTGTATCGTCTTCTGTAAGAGGATTTCAGATATGGACGGTAGAGCCAACTGGCGACAATGAGTTTAATGTAACCTACA 11279
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S. parauberis   GTGTAGACCAGCTCATTACAGAGGGAGAAAATACAAAGACCGTCCACTCTGCTTATATAGTGAGTGTCTATGTAGATGGTTCTGGAAATATGGTACTGGTTAAGAATCCGACCATTACCA 11398
S. agalatiae    GTGTAGACCAGCTCATTACAGAGGGAGAAAATACAAAGACCGTCCACTCTGCTTATATAGTGAGTGTCTATGTAGATGGTTCTGGAAATATGGTACTGGTTAAGAATCCGACCATTACCA 11398
pCF10           GTGTAGACCAGCTCATTACAGAGGGAGAAAATACAAAGACCGTCCACTCTGCTTATATAGTGAGTGTCTATGTAGATGGTTCTGGAAATATGGTACTGGTTAAGAATCCGACCATTACCA 11399
E. faecalis     GTGTAGACCAGCTCATTACAGAGGGAGAAAATACAAAGACCGTCCACTCTGCTTATATAGTGAGTGTCTATGTAGATGGTTCTGGAAATATGGTACTGGTTAAGAATCCGACCATTACCA 11399
S. suis         GTGTAGACCAGCTCATTACAGAGGGAGAAAATACAAAGACCGTCCACTCTGCTTATATAGTGAGTGTCTATGTAGATGGTTCTGGAAATATGGTACTGGTTAAGAATCCGACCATTACCA 11399
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S. parauberis   ACATACCTAAGAAATCAAGTTATAAACCAAAAGCCATTGAAAGTGAGGGGACGGTTGATTCCATTACAACCAATGAAATCAATGAGTTTTTAACGACGTTCTTCAAGCTCTATCCTACAG 11518
S. agalatiae    ACATACCTAAGAAATCAAGTTATAAACCAAAAGCCATTGAAAGTGAGGGGACGGTTGATTCCATTACAACCAATGAAATCAATGAGTTTTTAACGACGTTCTTCAAGCTCTATCCTACAG 11518
pCF10           ACATACCTAAGAAATCAAGTTATAAACCAAAAGCCATTGAAAGTGAGGGGACGGTTGATTCCATTACAACCAATGAAATCAATGAGTTTTTAACGACGTTCTTCAAGCTCTATCCTACAG 11519
E. faecalis     ACATACCTAAGAAATCAAGTTATAAACCAAAAGCCATTGAAAGTGAGGGGACGGTTGATTCCATTACAACCAATGAAATCAATGAGTTTTTAACGACGTTCTTCAAGCTCTATCCTACAG 11519
S. suis         ACATACCTAAGAAATCAAGTTATAAACCAAAAGCCATTGAAAGTGAGGGGACGGTTGATTCCATTACAACCAATGAAATCAATGAGTTTTTAACGACGTTCTTCAAGCTCTATCCTACAG 11519
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S. parauberis   CGACAGCCAGTGAACTTTCCTACTATGTGAATGACGGGATATTAAAACCAATCGGAAAAGAGTACATCTTTCAAGAACTGGTAAATCCTATTCACAATCGTAAGGATAATCAAGTCACGG 11638
S. agalatiae    CGACAGCCAGTGAACTTTCCTACTATGTGAATGACGGGATATTAAAACCAATCGGAAAAGAGTACATCTTTCAAGAACTGGTAAATCCTATTCACAATCGTAAGGATAATCAAGTCACGG 11638
pCF10           CGACAGCCAGTGAACTTTCCTACTATGTGAATGACGGGATATTAAAACCAATCGGAAAAGAGTACATCTTTCAAGAACTGGTAAATCCTATTCACAATCGTAAGGATAATCAAGTCACGG 11639
E. faecalis     CGACAGCCAGTGAACTTTCCTACTATGTGAATGACGGGATATTAAAACCAATCGGAAAAGAGTACATCTTTCAAGAACTGGTAAATCCTATTCACAATCGTAAGGATAATCAAGTCACGG 11639
S. suis         CGACAGCCAGTGAACTTTCCTACTATGTGAATGACGGGATATTAAAACCAATCGGAAAAGAGTACATCTTTCAAGAACTGGTAAATCCTATTCACAATCGTAAGGATAATCAAGTCACGG 11639
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S. parauberis   TATCGCTGACAGTGGAGTATATCGACCAGCAGACCAAAGCAACGCAGGTATCTCAATTTGATTTGGTACTTGAAAAGAACGGGAGTAATTGGAAGATTGTAAAATAACAAATATTGGTAC 11758
S. agalatiae    TATCGCTGACAGTGGAGTATATCGACCAGCAGACCAAAGCAACGCAGGTATCTCAATTTGATTTGGTACTTGAAAAGAACGGGAGTAATTGGAAGATTGTAAAATAACAAATATTGGTAC 11758
pCF10           TATCGCTGACAGTGGAGTATATCGACCAGCAGACCAAAGCAACGCAGGTATCTCAATTTGATTTGGTACTTGAAAAGAACGGGAGTAATTGGAAGATTGTAAAATAACAAATATTGGTAC 11759
E. faecalis     TATCGCTGACAGTGGAGTATATCGACCAGCAGACCAAAGCAACGCAGGTATCTCAATTTGATTTGGTACTTGAAAAGAACGGGAGTAATTGGAAGATTATAGAATAACAAATATTGGTAC 11759
S. suis         TATCGCTGACAGTGGAGTATATCGACCAGCAGACCAAAGCAACGCAGGTATCTCAATTTGATTTGGTACTTGAAAAGAACGGGAGTAATTGGAAGATTGTAAAATAACAAATATTGGTAC 11759
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S. parauberis   ATGATTACAGATACTTTGTAATCATGTACTCTTTTTGATAAAAAATTGGAGATTCCTTTACAAATATGCTCTTACGTGCTATTATTTAAGTGACTATTTAAAAGGAGTTAATAAATATGC 11878
S. agalatiae    ATGATTACAGATACTTTGTAATCATGTACTCTTTTTGATAAAAAATTGGAGATTCCTTTACAAATATGCTCTTACGTGCTATTATTTAAGTGACTATTTAAAAGGAGTTAATAAATATGC 11878
pCF10           ATGATTACAGATACTTTGTAATCATGTACTCTTTTTGATAAAAAATTGGAGATTCCTTTACAAATATGCTCTTACGTGCTATTATTTAAGTGACTATTTAAAAGGAGTTAATAAATATGC 11879
E. faecalis     ATTATTACAGCTATTTTGTAATCACGTACTCTCTTTGATAAAAAATTGGAGATTCCTTTACAAATATGCTCTTACGTGCTATTATTTAAGTATCTATTTAAAAGGAGTTAATAAATATGC 11879
S. suis         ATGATTACAGATACTTTGTAATCATGTACTCTTTTTGATAAAAAATTGGAGATTCCTTTACAAATACGCTCTTACGTGCTATTATTTAAGTGACTATTTAAAAGGAGTTAATAAATATGC 11879
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S. parauberis   GGCAAGGTATTCTTAAATAAACTGTCAATTTGATAGCGGGAACAAATAATTAGATGTCCTTTTTTAGGAGGGCTTAGTTTTTTGTACCCAGTTTAAGAATACCTTTATCATGTGATTCTA 11998
S. agalatiae    GGCAAGGTATTCTTAAATAAACTGTCAATTTGATAGCGGGAACAAATAATTAGATGTCCTTTTTTAGGAGGGCTTAGTTTTTTGTACCCAGTTTAAGAATACCTTTATCATGTGATTCTA 11998
pCF10           GGCAAGGTATTCTTAAATAAACTGTCAATTTGATAGCGGGAACAAATAATTAGATGTCCTTTTTTAGGAGGGCTTAGTTTTTTGTACCCAGTTTAAGAATACCTTTATCATGTGATTCTA 11999
E. faecalis     GGCAAGGTATTATTAAATAAACTGTCAATTTGATAGCGGGAACAAATAATTGGATGTCCTTTTTTAGGAGGGCTTAGTTTTTTGTACCCAGTTTAAGAATACCTTTATCATGTGATTCTA 11999
S. suis         GGCAAGGTATTCTTAAATAAACTGTCAATTTGATAGCAGGAACAAATAATTAGATGTCCTTTTTTAGGAGGGCTTAGTTTTTTGTACCCAGTTTAAGAATACCTTTATCATGTGATTCTA 11999
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S. parauberis   AAGTATCCAGAGAATATCTGTATGCTTTGTATACCTATGGTTATGCATAAAAATCCCAGTGATAAAAGTATTTATCACTGGGATTTTTATGCCCTTTTGGGTTTTTGAATGGAGGAAAAT 12118
S. agalatiae    AAGTATCCAGAGAATATCTGTATGCTTTGTATACCTATGGTTATGCATAAAAATCCCAGTGATAAAAGTATTTATCACTGGGATTTTTATGCCCTTTTGGGTTTTTGAATGGAGGAAAAT 12118
pCF10           AAGTATCCGGAGAATATCTGTATGCTTTGTATACCTATGGTTATGCATAAAAATCCCAGTGATAAAAGTATTTATCACTGGGATTTTTATGCCCTTTTGGGTTTTTGAATGGAGGAAAAT 12119
E. faecalis     AAGTATCCGGAGAATATCTGTATGCTTTGTATGCCTATGGTTATGCATAAAAATCCCAGTGATAAGAGTATTTATCACTGGGATTTTTATGCCCTTTTGGGCTTTTGAATGGAGGAAAAT 12119
S. suis         AAGTATCCAGAGAATATCTGTATGCTTTGTATGCCTATGGTTATGCATAAAAATCCCAGTGATAAAAGTATTTATCACTGGGATTTTTATGCCCTTTTGGGTTTTTGAATGGAGGAAAAT 12119
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S. parauberis   CACATGAAAATTATTAATATTGGAGTTTTAGCTCATGTTGATGCAGGAAAAACTACCTTAACAGAAAGCTTATTATATAACAGTGGAGCGATTACAGAATTAGGAAGCGTGGACAAAGGT 12238
S. agalatiae    CACATGAAAATTATTAATATTGGAGTTTTAGCTCATGTTGATGCAGGAAAAACTACCTTAACAGAAAGCTTATTATATAACAGTGGAGCGATTACAGAATTAGGAAGCGTGGACAAAGGT 12238
pCF10           CACATGAAAATTATTAATATTGGAGTTTTAGCTCATGTTGATGCAGGAAAAACTACCTTAACAGAAAGCTTATTATATAACAGTGGAGCGATTACAGAATTAGGAAGCGTGGACAAAGGT 12239
E. faecalis     CACATGAAAATTATTAATATTGGAGTTTTAGCTCATGTTGATGCAGGAAAAACTACCTTAACAGAAAGCTTATTATATAACAGTGGAGCGATTACAGAATTAGGAAGCGTGGACAAAGGT 12239
S. suis         CACATGAAAATTATTAATATTGGAGTTTTAGCTCATGTTGATGCAGGAAAAACTACCTTAACAGAAAGCTTATTATATAACAGTGGAGCGATTACAGAATTAGGAAGCGTGGACAAAGGT 12239
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S. parauberis   ACAACGAGGACGGATAATACGCTTTTAGAACGTCAGAGAGGAATTACAATTCAGACAGGAATAACCTCTTTTCAGTGGGAAAATACGAAGGTGAACATCATAGACACGCCAGGACATATG 12358
S. agalatiae    ACAACGAGGACGGATAATACGCTTTTAGAACGTCAGAGAGGAATTACAATTCAGACAGGAATAACCTCTTTTCAGTGGGAAAATACGAAGGTGAACATCATAGACACGCCAGGACATATG 12358
pCF10           ACAACGAGGACGGATAATACGCTTTTAGAACGTCAGAGAGGAATTACAATTCAGACAGGAATAACCTCTTTTCAGTGGGAAAATACGAAGGTGAACATCATAGACACGCCAGGACATATG 12359
E. faecalis     ACAACGAGGACGGATAATACGCTTTTAGAACGTCAGAGAGGAATTACAATTCAGACAGGAATAACCTCTTTTCAGTGGGAAAATACGAAGGTGAACATCATAGACACGCCAGGACATATG 12359
S. suis         ACAACGAGGACGGATAATACGCTTTTAGAACGTCAGAGAGGAATTACAATTCAGACAGGAATAACCTCTTTTCAGTGGGAAAATACGAAGGTGAACATCATAGACACGCCAGGACATATG 12359
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S. parauberis   GATTTCTTAGCAGAAGTATATCGTTCATTATCAGTTTTAGATGGGGCAATTCTACTGATTTCTGCAAAAGATGGCGTACAAGCACAAACTCGTATATTATTTCATGCACTTAGGAAAATG 12478
S. agalatiae    GATTTCTTAGCAGAAGTATATCGTTCATTATCAGTTTTAGATGGGGCAATTCTACTGATTTCTGCAAAAGATGGCGTACAAGCACAAACTCGTATATTATTTCATGCACTTAGGAAAATG 12478
pCF10           GATTTCTTAGCAGAAGTATATCGTTCATTATCAGTTTTAGATGGGGCAATTCTACTGATTTCTGCAAAAGATGGCGTACAAGCACAAACTCGTATATTATTTCATGCACTTAGGAAAATG 12479
E. faecalis     GATTTCTTAGCAGAAGTATATCGTTCATTATCAGTTTTAGATGGGGCAATTCTACTGATTTCTGCAAAAGATGGCGTACAAGCACAAACTCGTATATTATTTCATGCACTTAGGAAAATG 12479
S. suis         GATTTCTTAGCAGAAGTATATCGTTCATTATCAGTTTTAGATGGAGCAATTCTACTGATTTCTGCAAAAGATGGCGTACAAGCACAAACTCGTATATTATTTCATGCACTTAGGAAAATG 12479
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S. parauberis   GGGATTCCCACAATCTTTTTTATCAATAAGATTGACCAAAATGGAATTGATTTATCAACGGTTTATCAGGATATTAAAGAGAAACTTTCTGCCGAAATTGTAATCAAACAGAAGGTAGAA 12598
S. agalatiae    GGGATTCCCACAATCTTTTTTATCAATAAGATTGACCAAAATGGAATTGATTTATCAACGGTTTATCAGGATATTAAAGAGAAACTTTCTGCCGAAATTGTAATCAAACAGAAGGTAGAA 12598
pCF10           GGGATTCCCACAATCTTTTTTATCAATAAGATTGACCAAAATGGAATTGATTTATCAACGGTTTATCAGGATATTAAAGAGAAACTTTCTGCCGAAATTGTAATCAAACAGAAGGTAGAA 12599
E. faecalis     GGGATTCCCACAATCTTTTTTATCAATAAGATTGACCAAAATGGAATTGATTTATCAACGGTTTATCAGGATATTAAAGAGAAACTTTCTGCCGAAATTGTAATCAAACAGAAGGTAGAA 12599
S. suis         GGGATTCCCACAATCTTTTTTATCAATAAGATTGACCAAAATGGAATTGATTTATCAACGGTTTATCAGGATATTAAAGAGAAACTTTCTATGGAAATTATAATCAAACAGAAAGTAGAG 12599
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S. parauberis   CTGTATCCTAATATGTGTGTGACGAACTTTACCGAATCTGAACAATGGGATACGGTAATAGAGGGAAACGATGACCTTTTAGAGAAATATATGTCCGGTAAATCATTAGAAGCATTGGAA 12718
S. agalatiae    CTGTATCCTAATATGTGTGTGACGAACTTTACCGAATCTGAACAATGGGATACGGTAATAGAGGGAAACGATGACCTTTTAGAGAAATATATGTCCGGTAAATCATTAGAAGCATTGGAA 12718
pCF10           CTGTATCCTAATATGTGTGTGACGAACTTTACCGAATCTGAACAATGGGATACGGTAATAGAGGGAAACGATGACCTTTTAGAGAAATATATGTCCGGTAAATCATTAGAAGCATTGGAA 12719
E. faecalis     CTGTATCCTAATGTGTGTGTGACGAACTTTACCGAATCTGAACAATGGGATACGGTAATAGAGGGAAACGATGACCTTTTAGAGAAATATATGTCCGGTAAATCATTAGAAGCATTGGAA 12719
S. suis         CTGCACCCTAATATGTGTGTGATGAGCTGTACGGAACCTGAGCAATGGGATGTGGTAATAGAAGGAAATGATGACCTTTTAGAGAAATATATGTCCGGTAAATCATTAGAAGCATTAGAA 12719
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S. parauberis   CTCGAACAAGAGGAAAGCATAAGATTTCATAATTGTTCCCTGTTCCCTGTTTATCACGGAAGTGCAAAAAACAATATAGGGATTGATAACCTTATAGAAGTGATTACGAATAAATTTTAT 12838
S. agalatiae    CTCGAACAAGAGGAAAGCATAAGATTTCATAATTGTTCCCTGTTCCCTGTTTATCACGGAAGTGCAAAAAACAATATAGGGATTGATAACCTTATAGAAGTGATTACGAATAAATTTTAT 12838
pCF10           CTCGAACAAGAGGAAAGCATAAGATTTCATAATTGTTCCCTGTTCCCTGTTTATCACGGAAGTGCAAAAAACAATATAGGGATTGATAACCTTATAGAAGTGATTACGAATAAATTTTAT 12839
E. faecalis     CTCGAACAAGAGGAAAGCATAAGATTTCAGAATTGTTCTCTGTTCCCTCTTTATCATGGAAGTGCAAAAAGTAATATAGGGATTGATAACCTTATAGAAGTTATTACTAATAAATTTTAT 12839
S. suis         CTCGAACAAGAGGAAATCAGAAGATTTCAGAATTGCTCCTTGTACCCTGTTTATCATGGAAGCGCAAAAAGCAACATAGGGATTGAGCAGCTTATAGAAGTGATAACGAATAAATTTTAT 12839
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S. parauberis   TCATCAACACATCGAGGTCCGTCTGAACTTTGCGGAAATGTTTTCAAAATTGAATATACAAAAAAAAGACAACGTCTTGCATATATACGCCTTTATAGTGGAGTACTACATTTACGAGAT 12958
S. agalatiae    TCATCAACACATCGAGGTCCGTCTGAACTTTGCGGAAATGTTTTCAAAATTGAATATACAAAAAAAAGACAACGTCTTGCATATATACGCCTTTATAGTGGAGTACTACATTTACGAGAT 12958
pCF10           TCATCAACACATCGAGGTCCGTCTGAACTTTGCGGAAATGTTTTCAAAATTGAATATACAAAAAAAAGACAACGTCTTGCATATATACGCCTTTATAGTGGAGTACTACATTTACGAGAT 12959
E. faecalis     TCATCAACACATCGAGGTCCGTCTGAACTTTGCGGAAATGTTTTCAAAATTGAATATACAAAAAAAAGACAACGTCTTGCATATATACGCCTTTATAGTGGAGTACTACATTTACGAGAT 12959
S. suis         TCATCAACATACAGAAAGAAGTCTGAACTTTGCGGAAATGTCTTCAAAATTGAATATTCGGAAGAAAGACAACGTCTTGCATATGTACGCCTTTATGGCGGAATCCTGCATTTGCGGGAT 12959
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S. parauberis   TCGGTTAGAGTATCAGAAAAAGAAAAAATAAAAGTTACAGAAATGTATACTTCAATAAATGGTGAATTATGTAAGATTGATAGAGCTTATTCTGGAGAAATTGTTATTTTGCAAAATGAG 13078
S. agalatiae    TCGGTTAGAGTATCAGAAAAAGAAAAAATAAAAGTTACAGAAATGTATACTTCAATAAATGGTGAATTATGTAAGATTGATAGAGCTTATTCTGGAGAAATTGTTATTTTGCAAAATGAG 13078
pCF10           TCGGTTAGAGTATCAGAAAAAGAAAAAATAAAAGTTACAGAAATGTATACTTCAATAAATGGTGAATTATGTAAGATTGATAGAGCTTATTCTGGAGAAATTGTTATTTTGCAAAATGAG 13079
E. faecalis     TCGGTTAGAGTATCAGAAAAAGAAAAAATAAAAGTTACAGAAATGTATACTTCAATAAATGGTGAATTATGTAAGATTGATAGAGCTTATTCTGGAGAAATTGTTATTTTGCAAAATGAG 13079
S. suis         TCGGTTAGAATATCGGAAAAGGAAAAAATAAAAATTACAGAAATGTATACTTCAATAAATGGTGAATTATGTAAGATTGATAAGGCTTATTCCGGGGAAATTGTTATTTTGCAAAATGAG 13079
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S. parauberis   TTTTTGAAGTTAAATAGTGTTCTTGGAGATACAAAACTATTGCCACAGAGAAAAAAGATTGAAAATCCGCACCCTCTACTACAAACAACTGTTGAACCGAGTAAACCTGAACAGAGAGAA 13198
S. agalatiae    TTTTTGAAGTTAAATAGTGTTCTTGGAGATACAAAACTATTGCCACAGAGAAAAAAGATTGAAAATCCGCACCCTCTACTACAAACAACTGTTGAACCGAGTAAACCTGAACAGAGAGAA 13198
pCF10           TTTTTGAAGTTAAATAGTGTTCTTGGAGATACAAAACTATTGCCACAGAGAAAAAAGATTGAAAATCCGCACCCTCTACTACAAACAACTGTTGAACCGAGTAAACCTGAACAGAGAGAA 13199
E. faecalis     TTTTTGAAGTTAAATAGTGTTCTTGGAGATACAAAACTATTGCCACAGAGAAAAAAGATTGAAAATCCGCACCCTCTACTACAAACAACTGTTGAACCGAGTAAACCTGAACAGAGAGAA 13199
S. suis         TTTTTGAAGCTAAATAGTGTTCTTGGAGATACAAAGCTATTGCCACAGAGAGAGAGAATTGAAAATCCGCTCCCTCTGCTGCAAACAACTGTTGAACCGAGCAAACCTCAACAAAGGGAA 13199
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S. parauberis   ATGTTGCTTGATGCCCTTTTGGAAATCTCAGATAGTGATCCGCTTCTACGATATTACGTGGATTCTACGACACATGAAATTATACTTTCTTTCTTAGGGAAAGTACAAATGGAAGTGATT 13318
S. agalatiae    ATGTTGCTTGATGCCCTTTTGGAAATCTCAGATAGTGATCCGCTTCTACGATATTACGTGGATTCTACGACACATGAAATTATACTTTCTTTCTTAGGGAAAGTACAAATGGAAGTGATT 13318
pCF10           ATGTTGCTTGATGCCCTTTTGGAAATCTCAGATAGTGATCCGCTTCTACGATATTACGTGGATTCTACGACACATGAAATTATACTTTCTTTCTTAGGGAAAGTACAAATGGAAGTGATT 13319
E. faecalis     ATGTTGCTTGATGCCCTTTTGGAAATCTCAGATAGTGATCCGCTTCTACGATATTACGTGGATTCTACGACACATGAAATTATACTTTCTTTCTTAGGGAAAGTACAAATGGAAGTGATT 13319
S. suis         ATGTTACTTGATGCACTTTTAGAAATCTCCGACAGTGACCCGCTTCTACAATATTATGTGGATTCTACGACACATGAAATTATACTTTCTTTCTTAGGGAAAGTACAAATGGAAGTGATT 13319
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S. parauberis   AGTGCACTGTTGCAAGAAAAGTATCATGTGGAGATAGAACTAAAAGAGCCTACAGTCATTTATATGGAGAGACCGTTAAAAAATGCAGAATATACCATTCACATCGAAGTGCCGCCAAAT 13438
S. agalatiae    AGTGCACTGTTGCAAGAAAAGTATCATGTGGAGATAGAACTAAAAGAGCCTACAGTCATTTATATGGAGAGACCGTTAAAAAATGCAGAATATACCATTCACATCGAAGTGCCGCCAAAT 13438
pCF10           AGTGCACTGTTGCAAGAAAAGTATCATGTGGAGATAGAACTAAAAGAGCCTACAGTCATTTATATGGAGAGACCGTTAAAAAATGCAGAATATACCATTCACATCGAAGTGCCGCCAAAT 13439
E. faecalis     AGTGCACTGTTGCAAGAAAAGTATCATGTGGAGATAGAACTAAAAGAGCCTACAGTCATTTATATGGAGAGACCGTTAAAAAATGCAGAATATACCATTCACATCGAAGTGCCGCCAAAT 13439
S. suis         AGTGCACTGTTGCAAGAAAAGTATCATGTGGAGATAGAACTAAAAGAGCCTACAGTCATTTATATGGAGAGACCGTTAAAAAATGCAGAATATACCATTCACATCGAAGTGCCGCCAAAT 13439
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S. parauberis   CCTTTCTGGGCTTCCATTGGTTTATCTGTATCACCGCTTCCGTTGGGAAGTGGAATGCAGTATGAGAGCTCGGTTTCTCTTGGATACTTAAATCAATCATTTCAAAATGCAGTTATGGAA 13558
S. agalatiae    CCTTTCTGGGCTTCCATTGGTTTATCTGTATCACCGCTTCCGTTGGGAAGTGGAATGCAGTATGAGAGCTCGGTTTCTCTTGGATACTTAAATCAATCATTTCAAAATGCAGTTATGGAA 13558
pCF10           CCTTTCTGGGCTTCCATTGGTTTATCTGTATCACCGCTTCCGTTGGGAAGTGGAATGCAGTATGAGAGCTCGGTTTCTCTTGGATACTTAAATCAATCATTTCAAAATGCAGTTATGGAA 13559
E. faecalis     CCTTTCTGGGCTTCCATTGGTTTATCTGTATCACCGCTTCCGTTGGGAAGTGGAATGCAGTATGAGAGCTCGGTTTCTCTTGGATACTTAAATCAATCATTTCAAAATGCAGTTATGGAA 13559
S. suis         CCTTTCTGGGCTTCCATTGGTTTATCTGTATCACCGCTTCCGTTGGGAAGTGGAATGCAGTATGAGAGCTCGGTTTCTCTTGGATACTTAAATCAATCATTTCAAAATGCAGTTATGGAA 13559
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S. parauberis   GGGATACGCTATGGTTGCGAACAAGGATTATATGGTTGGAATGTGACGGATTGTAAAATCTGTTTTAAGTATGGCTTATACTATAGCCCTGTTAGTACCCCAGCAGATTTTCGGATGCTT 13678
S. agalatiae    GGGATACGCTATGGTTGCGAACAAGGATTATATGGTTGGAATGTGACGGATTGTAAAATCTGTTTTAAGTATGGCTTATACTATAGCCCTGTTAGTACCCCAGCAGATTTTCGGATGCTT 13678
pCF10           GGGATACGCTATGGTTGCGAACAAGGATTATATGGTTGGAATGTGACGGATTGTAAAATCTGTTTTAAGTATGGCTTATACTATAGCCCTGTTAGTACCCCAGCAGATTTTCGGATGCTT 13679
E. faecalis     GGGATACGCTATGGTTGTGAACAAGGATTGTATGGTTGGAATGTGACGGACTGTAAAATCTGTTTTAAGTATGGCTTATACTATAGCCCTGTTAGTACCCCAGCAGATTTTCGGATGCTT 13679
S. suis         GGGATACGCTATGGTTGCGAACAAGGATTATATGGTTGGAATGTGACGGATTGTAAAATCTGTTTTAAGTATGGCTTATACTATAGCCCTGTTAGTACCCCAGCAGATTTTCGGATGCTT 13679
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S. parauberis   GCTCCTATTGTATTGGAACAAGTCTTAAAAAAAGCTGGAACAGAATTGTTAGAGCCATATCTTAGTTTTAAAATTTATGCGCCACAGGAATATCTTTCACGAGCATACAACGATGCTCCT 13798
S. agalatiae    GCTCCTATTGTATTGGAACAAGTCTTAAAAAAAGCTGGAACAGAATTGTTAGAGCCATATCTTAGTTTTAAAATTTATGCGCCACAGGAATATCTTTCACGAGCATACAACGATGCTCCT 13798
pCF10           GCTCCTATTGTATTGGAACAAGTCTTAAAAAAAGCTGGAACAGAATTGTTAGAGCCATATCTTAGTTTTAAAATTTATGCGCCACAGGAATATCTTTCACGAGCATACAACGATGCTCCT 13799
E. faecalis     GCTCCTATTGTATTGGAACAAGTCTTAAAAAAAGCTGGAACAGAATTGTTAGAGCCATATCTTAGTTTTAAAATTTATGCGCCACAGGAATATCTTTCACGAGCATACAACGATGCTCCT 13799
S. suis         GCTCCTATTGTATTGGAACAAGTCTTAAAAAAAGCTGGAACAGAATTGTTAGAGCCATATCTTAGTTTTAAAATTTATGCGCCACAGGAATATCTTTCACGAGCATACAACGATGCTCCT 13799
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S. parauberis   AAATATTGTGCGAACATCGTAGACACTCAATTGAAAAATAATGAGGTCATTCTTAGTGGAGAAATCCCTGCTCGGTGTATTCAAGAATATCGTAGTGATTTAACTTTCTTTACAAATGGA 13918
S. agalatiae    AAATATTGTGCGAACATCGTAGACACTCAATTGAAAAATAATGAGGTCATTCTTAGTGGAGAAATCCCTGCTCGGTGTATTCAAGAATATCGTAGTGATTTAACTTTCTTTACAAATGGA 13918
pCF10           AAATATTGTGCGAACATCGTAGACACTCAATTGAAAAATAATGAGGTCATTCTTAGTGGAGAAATCCCTGCTCGGTGTATTCAAGAATATCGTAGTGATTTAACTTTCTTTACAAATGGA 13919
E. faecalis     AAATATTGTGCGAACATCGTAGACACTCAATTGAAAAATAATGAGGTCATTCTTAGTGGAGAAATCCCTGCTCGGTGTATTCAAGAATATCGTAGTGATTTAACTTTCTTTACAAATGGA 13919
S. suis         AAATATTGTGCGAACATCGTAGACACTCAATTGAAAAATAATGAGGTCATTCTTAGTGGAGAAATCCCTGCTCGGTGTATTCAAGAATATCGTAGTGATTTAACTTTCTTTACAAATGGA 13919
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S. parauberis   CGTAGTGTTTGTTTAACAGAGTTAAAAGGGTACCATGTTACTACCGGTGAACCTGTTTGCCAGCCCCGTCGTCCAAATAGTCGGATAGATAAAGTACGATATATGTTCAATAAAATAACT 14038
S. agalatiae    CGTAGTGTTTGTTTAACAGAGTTAAAAGGGTACCATGTTACTACCGGTGAACCTGTTTGCCAGCCCCGTCGTCCAAATAGTCGGATAGATAAAGTACGATATATGTTCAATAAAATAACT 14038
pCF10           CGTAGTGTTTGTTTAACAGAGTTAAAAGGGTACCATGTTACTACCGGTGAACCTGTTTGCCAGCCCCGTCGTCCAAATAGTCGGATAGATAAAGTACGATATATGTTCAATAAAATAACT 14039
E. faecalis     CGTAGTGTTTGTTTAACAGAGTTAAAAGGGTACCATGTTACTACCGGTGAACCTGTTTGCCAGCCCCGTCGTCCAAATAGTCGGATAGATAAAGTACGATATATGTTCAATAAAATAACT 14039
S. suis         CGTAGTGTTTGTTTAACAGAGTTAAAAGGGTACCATGTTACTACCGGTGAACCTGTTTGCCAGCCCCGTCGTCCAAATAGTCGGATAGATAAAGTACGATATATGTTCAATAAAATAACT 14039
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S. parauberis   TAGTGTATTTTATGTTGTTATATAAATATGGTTTCTTGTTAAATAAGATGAAATATTTTTTAATAAAGATTTGAATTAAAGTGTAAAGGAGGAGATAGTTATTATAAACTACAAGTGGAT 14158
S. agalatiae    TAGTGTATTTTATGTTGTTATATAAATATGGTTTCTTGTTAAATAAGATGAAATATTTTTTAATAAAGATTTGAATTAAAGTGTAAAGGAGGAGATAGTTATTATAAACTACAAGTGGAT 14158
pCF10           TAGTGTATTTTATGTTGTTATATAAATATGGTTTCTTGTTAAATAAGATGAAATATTTTTTAATAAAGATTTGAATTAAAGTGTAAAGGAGGAGATAGTTATTATAAACTACAAGTGGAT 14159
E. faecalis     TAGTGTATTTTATGTTGTTATATAAATATGGTTTCTTGTTAAATAAGATGAAATATTTTTTAATAAAGATTTGAATTAAAGTGTAAAGGAGGAGATAGTTATTATAAACTACAAGTGGAT 14159
S. suis         TAGTGTATTTTATGTTGTTATATAAATATGGTTTCTTGTTAAATAAGATGAAATATTTTTTAATAAAGATTTGAATTAAAGTGTAAAGGAGGAGATAGTTATTATAAACTACAAGTGGAT 14159
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S. parauberis   ATTGTGTCCTGTATGTGGAAATAAAACACGATTAAAGATAAGGGAAGATACTGAATTAAAAAAATTCCCCCTCTATTGTCCGAAATGCAGACAAGAAAATTTAATTGAAATAAAGCAGTT 14278
S. agalatiae    ATTGTGTCCTGTATGTGGAAATAAAACACGATTAAAGATAAGGGAAGATACTGAATTAAAAAAATTCCCCCTCTATTGTCCGAAATGCAGACAAGAAAATTTAATTGAAATAAAGCAGTT 14278
pCF10           ATTGTGTCCTGTATGTGGAAATAAAACACGATTAAAGATAAGGGAAGATACTGAATTAAAAAAATTCCCCCTCTATTGTCCGAAATGCAGACAAGAAAATTTAATTGAAATAAAGCAGTT 14279
E. faecalis     ATTGTGTCCTGTATGTGGAAATAAAACACGATTAAAGATAAGGGAAGATACTGAATTAAAAAAATTCCCCCTCTATTGTCCGAAATGCAGACAAGAAAATTTAATTGAAATAAAGCAGTT 14279
S. suis         ATTGTGTCCTGTATGTGGAAATAAAACACGATTAAAGATAAGGGAAGATACTGAATTAAAAAAATTCCCCCTCTATTGTCCGAAATGCAGACAAGAAAATTTAATTGAAATAAAGCAGTT 14279
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S. parauberis   CAAAGTAACTGTGATTACAGAGCCAGACGCAAAGACGCAGAGCCGATAAAATGAGATTAATACAATCTCATTTTATCGGCTCTTTCCGTTATGTATGGATTCTTTTAATTAGTCTTCGAT 14398
S. agalatiae    CAAAGTAACTGTGATTACAGAGCCAGACGCAAAGACGCAGAGCCGATAAAATGAGATTAATACAATCTCATTTTATCGGCTCTTTCCGTTATGTATGGATTCTTTTAATTAGTCTTCGAT 14398
pCF10           CAAAGTAACTGTGATTACAGAGCCAGACGCAAAGACGCAGAGCCGATAAAATGAGATTAATACAATCTCATTTTATCGGCTCTTTCCGTTATGTATGGATTCTTTTAATTAGTCTTCGAT 14399
E. faecalis     CAAAGTAACTGTGATTACAGAGCCAGACGCAAAGACGCAGAGCCGATAAAATGAGATTAATACAATCTCATTTTATCGGCTCTTTCCGTTATGTATGGATTCTTTTAATTAGTCTTCGAT 14399
S. suis         CAAAGTAACTGTGATTACAGAGCCAGACGCAAAGACGCAGAGCCGATAAAATGAGATTAATACAATCTCATTTTATCGGCTCTTTCCGTTATGTATGGATTCTTTTAATTAGTCTTCGAT 14399
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S. parauberis   GTTTCTTGCTTCGTTGATACCGCTGGCTAAAGATTCCATTAAGGATAGTTCTTTGTCTGTAAAGCTATCCATGTATTTCTCTATCTGTAATCGTCGGGTGCTTTTTACCAAGTTATTAGC 14518
S. agalatiae    GTTTCTTGCTTCGTTGATACCGCTGGCTAAAGATTCCATTAAGGATAGTTCTTTGTCTGTAAAGCTATCCATGTATTTCTCTATCTGTAATCGTCGGGTGCTTTTTACCAAGTTATTAGC 14518
pCF10           GTTTCTTGCTTCGTTGATACCGCTGGCTAAAGATTCCATTAAGGATAGTTCTTTGTCTGTAAAGCTATCCATGTATTTCTCTATCTGTAATCGTCGGGTGCTTTTTACCAAGTTATTAGC 14519
E. faecalis     GTTTCTTGCTTCGTTGATACCGCTGGCTAAAGATTCCATTAAGGATAGTTCTTTGTCTGTAAAGCTATCCATGTATTTCTCTATCTGTAATCGTCGGGTGCTTTTTACCAAGTTATTAGC 14519
S. suis         GTTTCTTGCTTCGTTAATACCGCTGGCTAAAGATTCCATTAAGGATAGTTCTTTGTCTGTAAAGCTATCCATGTATTTCTCTATCTGTAATCGTCGGGTGCTTTTTACCAAGTTATTAGC 14519
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S. parauberis  AGGTAAGAAAAATTCATCAACGGAAACATGAAGTAACGATACAAGGTCATAAAGAACTTGTATGCTGGGGTGTTGCCCTTTATTTTCAATATTAGTTAAGTACCGTGGGTCAATTTCAAT 14638
S. agalatiae   AGGTAAGAAAAATTCATCAACGGAAACATGAAGTAACGATACAAGGTCATAAAGAACTTGTATGCTGGGGTGTTGCCCTTTATTTTCAATATTAGTTAAGTACCGTGGGTCAATTTCAAT 14638
pCF10          AGGTAAGAAAAATTCATCAACGGAAACATGAAGTAACGATACAAGGTCATAAAGAACTTGTATGCTGGGGTGTTGCCCTTTATTTTCAATATTAGTTAAGTACCGTGGGTCAATTTCAAT 14639
E. faecalis    AGGTAAGAAAAATTCATCAACGGAAACATGAAGTAACGATACAAGGTCATAAAGAACTTGTATGCTGGGGTGTTGCCCTTTATTTTCAATATTAGTTAAGTACCGTGGGTCAATTTCAAT 14639
S. suis        AGGTAAGAAAAATTCATCAACGGAAACATGAAGTAACGATACAAGGTCATAAAGAACTTGTATGCTGGGGTGTTGCCCTTTATTTTCAATATTAGTTAAGTACCGTGGGTCAATTTCAAT 14639
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S. parauberis  CAATGCTCCCACTTGTTCACGAGTTAAACCTCGTTTCAATCGAGCTTCTTTAATGGCTAAACCAAAGGCTCTAAAATCATATTTATCTTCTTTTTTACGCATAGTAGACCACCTCTATAC 14758
S. agalatiae   CAATGCTCCCACTTGTTCACGAGTTAAACCTCGTTTCAATCGAGCTTCTTTAATGGCTAAACCAAAGGCTCTAAAATCATATTTATCTTCTTTTTTACGCATAGTAGACCACCTCTATAC 14758
pCF10          CAATGCTCCCACTTGTTCACGAGTTAAACCTCGTTTCAATCGAGCTTCTTTAATGGCTAAACCAAAGGCTCTAAAATCATATTTATCTTCTTTTTTACGCATAGTAGACCACCTCTATAC 14759
E. faecalis    CAATGCTCCCACTTGTTCACGAGTTAAACCTCGTTGCAATCGAGCTTCTTTAATGGCTAAACCAAAGGCTCTAAAATCATATTTATCTTCTTTTTTACGCATAGTAGACCACCTCTATAC 14759
S. suis        CAATGCTCCCACTTGTTCACGAGTTAAACCTCGTTTCAATCGAGCTTCTTTAATGGCTAAACCAAAGGCTCTAAAATCATATTTATCTTCTTTTTTACGCATAGTAGACCACCTCTATAC 14759
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S. parauberis  ATTTATTGTTCCTACTGAATTAAAAACAGGTATAGAAAAACGTGTTATATGGTTTATAGGTTTATATTTAATAAAAAGCACTACTAAACGCCAATAAAAAAAAACCGTTATATGGTAGTG 14878
S. agalatiae   ATTTATTGTTCCTACTGAATTAAAAACAGGTATAGAAAAACGTGTTATATGGTTTATAGGTTTATATTTAATAAAAAGCACTACTAAACGCCAATAAAAAAAAACCGTTATATGGTAGTG 14878
pCF10          ATTTATTGTTCCTACTGAATTAAAAACAGGTATAGAAAAACGTGTTATATGGTTTATAGGTTTATATTTAATAAAAAGCACTACTAAACGCCAATAAAAAAAAACCGTTATATGGTAGTG 14879
E. faecalis    ATTTATTGTTCCTACTGAATTAAAAACAGGTATAGAAAAACGTGTTATATGGTTTATAGGTTTATATTTAATAAAAAGCACTACTAAACGCCAATAAAAAAAAACCGTTATATGGTAGTG 14879
S. suis        ATTTATTGTTCCTACTGAATTAAAAACAGGTATAGAAAAACGTGTTATATGGTTTATAGGTTTATATTTAATAAAAAGCACTACTAAACGCCAATAAAAAAAAACCGTTATATGGTAGTG 14879
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S. parauberis  CTATTTACGCTGTTAAAATATTGTATATTACTTCCAAATGGCGGTTTGTTGGAGGTCAACGTCGCCATGAAGTACATCATATACAATAAATTTCCTTACATTGGGTTCTTGTCAAAAAAA 14998
S. agalatiae   CTATTTACGCTGTTAAAATATTGTATATTACTTCCAAATGGCGGTTTGTTGGAGGTCAACGTCGCCATGAAGTACATCATATACAATAAATTTCCTTACATTGGGTTATTGTCAAAAAAA 14998
pCF10          CTATTTACGCTGTTAAAATATTGTATATTACTTCCAAATGGCGGTTTGTTGGAGGTCAACGTCGCCATGAAGTACATCATATACAATAAATTTCCTTACATTGGGTTCTTGTCAAAAAAA 14999
E. faecalis    CTATTTACGCTGTTAAAATATTGTATATTACTTCCAAATGGCGGTTTGTTGGAGGTCAACGTCGCCATGAAGTACATCATATACAATAAATTTCCTTACATTGGGTTCTTGTCAAAAAAA 14999
S. suis        CTATTTACGCTGTTAAAATATTGTATATTACTTCCAAATGGCGGTTTGTTGGAGGTCAACGTCGCCATGAAGTACATCATATACAATAAATTTCCTTACATTGGGTTCTTGTCAAAAAAA 14999
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S. parauberis  GTCGTCTATCTGCAATAGATAAGTACGTCCACCAATGTGGTTTTATAAATCATATAGATAGAATAACAGAAGCATGTAAACAGAGAAATAAATCTGTTTATATGCTTTTTTGGCTATTCA 15118
S. agalatiae   GTCGTCTATCTGCAATAGATAAGTACGTCCACCAATGTGGTTTTATAAATCATATAGATAGAATAACAGAAGCATGTAAACAGAGAAATAAATCTGTTTATATGCTTTTTTGGCTATTCA 15118
pCF10          GTCGTCTATCTGCAATAGATAAGTACGTCCACCAATGTGGTTTTATAAATCATATAGATAGAATAACAGAAGCATGTAAACAGAGAAATAAATCTGTTTATATGCTTTTTTGGCTATTCA 15119
E. faecalis    GTCGTCTATCTGCAATAGATAAGTACGTCCACCAATGTGGTTTTATAAATCATATAGATAGAATAACAGAAGCATGTAAACAGAGAAATAAATCTGTTTATATGCTTTTTTGGCTATTCA 15119
S. suis        GTCGTCTATCTGCAATAGATAAGTACGTCCACCAATGTGGTTTTATAAATCATATAGATAGAATAACAGAAGCATGTAAACAGAGAAATAAATCTGTTTATATGCTTTTTTGGCTATTCA 15119
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S. parauberis  GAACTTTTTTACAAAGTTTATTTATCAGTAATGCAACAAATCCCCCTTTCACATTGGGACTAAGAGTGAAAGGAGATAAACGAGCAAGGCTCACTTCCTTTCCTAGACAGAAAGGGGGTG 15238
S. agalatiae   GAACTTTTTTACAAAGTTTATTTATCAGTAATGCAACAAATCCCCCTTTCACATTGGGACTAAGAGTGAAAGGAGATAAACGAGCAAGGCTCACTTCCTTTCCTAGACAGAAAGGGGGTG 15238
pCF10          GAACTTTTTTACAAAGTTTATTTATCAGTAATGCAACAAATCCCCCTTTCACATTGGGACTAAGAGTGAAAGGAGATAAACGAGCAAGGCTCACTTCCTTTCCTAGACAGAAAGGGGGTG 15239
E. faecalis    GAACTTTTTTACAAAGTTTATTTATCAGTAATGCAACAAATCCCCCTTTCACATTGGGACTAAGAGTGAAAGGAGATAAACGAGCAAGGCTCACTTCCTTTCCTAGACAGAAAGGGGGTG 15239
S. suis        GAACTTTTTTACAAAGTTTATTTATCAGTAATGCAACAAATCCCC-TTTCACATTGGGACTAAGAGTGAAAGGAGATAAACGAGCAAGGCTCACTTCCTTTCCTAGACAGAAAGGGGGTG 15238
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S. parauberis  AGAAACATGAAACCATCTTCTTTTCAGACCACAATAGAAAATCAGTTTGACTATATCTGTAAACGTGCTATGGAAGACGAGCGAAAGAATTATATGCTTTATCTTTCAAGGATTGCAAAG 15358
S. agalatiae   AGAAACATGAAACCATCTTCTTTTCAGACCACAATAGAAAATCAGTTTGACTATATCTGTAAACGTGCTATGGAAGACGAGCGAAAGAATTATATGCTTTATCTTTCAAGGATTGCAAAG 15358
pCF10          AGAAACATGAAACCATCTTCTTTTCAGACCACAATAGAAAATCAGTTTGACTATATCTGTAAACGTGCTATGGAAGACGAGCGAAAGAATTATATGCTTTATCTTTCAAGGATTGCAAAG 15359
E. faecalis    AGAAACATGAAACCATCTTCTTTTCAGACCACAATAGAAAATCAGTTTGACTATATCTGTAAACGTGCTATGGAAGACGAGCGAAAGAATTATATGCTTTATCTTTCAAGGATTGCAAAG 15359
S. suis        AGAAACATGAAACCATCTTCTTTTCAGACCACAATAGAAAATCAGTTTGACTATATCTGTAAACGTGCTATGGAAGACGAGCGAAAGAATTATATGCTTTATCTTTCAAGGATTGCAAAG 15358
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S. parauberis  CGTGAGGTGTCCTTTTCGGATGTTGGCGATTATCTTGTTAGCCAGTTTGCGACAACAGATAACTATTCAACTGACTTTCAGATTTTTACACTCAATGGGTTATCAGTAGGCGTTGAAAAT 15478
S. agalatiae   CGTGAGGTGTCCTTTTCGGATGTTGGCGATTATCTTGTTAGCCAGTTTGCGACAACAGATAACTATTCAACTGACTTTCAGATTTTTACACTCAATGGGTTATCAGTAGGCGTTGAAAAT 15478
pCF10          CGTGAGGTGTCCTTTTCGGATGTTGGCGATTATCTTGTTAGCCAGTTTGCGACAACAGATAACTATTCAACTGACTTTCAGATTTTTACACTCAATGGGTTATCAGTAGGCGTTGAAAAT 15479
E. faecalis    CGTGAGGTGTCCTTTTCGGATGTTGGCGATTATCTTGTTAGCCAGTTTGCGACAACAGATAACTATTCAACTGACTTTCAGATTTTTACACTCAATGGGTTATCAGTAGGCGTTGAAAAT 15479
S. suis        CGTGAGGTGTCCTTTTCGGATGTTGGCGATTATCTTGTTAGCCAGTTTGCGACAACAGATAACTATTCAACTGACTTTCAGATTTTTACACTCAATGGGTTATCAGTAGGCGTTGAAAAT 15478
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S. parauberis  GATTTGTTGAGTGAAGCATTACGTGAGTTGCCAGACAAGAAACGTGAAATTCTACTGCTGTTTTACTTTATGGACATGAGCGATTCAGAAATTGCAGACCTGTTGAAATTGAACCGTTCT 15598
S. agalatiae   GATTTGTTGAGTGAAGCATTACGTGAGTTGCCAGACAAGAAACGTGAAATTCTACTGCTGTTTTACTTTATGGACATGAGCGATTCAGAAATTGCAGACCTGTTGAAATTGAACCGTTCT 15598
pCF10          GATTTGTTGAGTGAAGCATTACGTGAGTTGCCAGACAAGAAACGTGAAATTCTACTGCTGTTTTACTTTATGGACATGAGCGATTCAGAAATTGCAGACCTGTTGAAATTGAACCGTTCT 15599
E. faecalis    GATTTGTTGAGTGAAGCATTACGTGAGTTGCCAGACAAGAAACGTGAAATTCTACTGCTGTTTTACTTTATGGACATGAGCGATTCAGAAATTGCAGACCTGTTGAAATTGAACCGTTCT 15599
S. suis        GATTTGTTGAGTGAAGCATTACGTGAGTTGCCAGACAAGAAACGTGAAATTCTACTGCTGTTTTACTTTATGGACATGAGCGATTCAGAAATTGCAGACCTGTTGAAATTGAACCGTTCT 15598
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S. parauberis  ACTGTCTATCGGCATAGAACCAGTGGACTAGCCTTAATTAAAAAGTTTATGGAGGAATTTGAAGAATGAAAACACAATATCCTATGATTCCCTTTCCTCTCATTGTAAAGGCAACAGATG 15718
S. agalatiae   ACTGTCTATCGGCATAGAACCAGTGGACTAGCCTTAATTAAAAAGTTTATGGAGGAATTTGAAGAATGAAAACACAATATCCTATGATTCCCTTTCCTCTCATTGTAAAGGCAACAGATG 15718
pCF10          ACTGTCTATCGGCATAGAACCAGTGGACTAGCCTTAATTAAAAAGTTTATGGAGGAATTTGAAGAATGAAAACACAATATCCTATGATTCCCTTTCCTCTCATTGTAAAGGCAACAGATG 15719
E. faecalis    ACTGTCTATCGGCATAGAACCAGTGGACTAGCCTTAATTAAAAAGTTTATGGAGGAATTTGAAGAATGAAAACACAATATCCTATGATTCCCTTTCCTCTCATTGTAAAGGCAACAGATG 15719
S. suis        ACTGTCTATCGGCATAGAACCAGTGGACTAGCCTTAATTAAAAAGTTTATGGAGGAATTTGAAGAATGAAAACACAATATCCTATGATTCCCTTTCCTCTCATTGTAAAGGCAACAGATG 15718
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S. parauberis  GCGATACCGAAGCGATTAACCAGATTCTACATCATTACAGAGGGTACATAACGAAGCGTTCCCTACGACTTATGAAAGATGAATATGGCAATCAAAGTATGGTCGTTGATGAAGTCTTAC 15838
S. agalatiae   GCGATACCGAAGCGATTAACCAGATTCTACATCATTACAGAGGGTACATAACGAAGCGTTCCCTACGACTTATGAAAGATGAATATGGCAATCAAAGTATGGTCGTTGATGAAGTCTTAC 15838
pCF10          GCGATACCGAAGCGATTAACCAGATTCTACATCATTACAGAGGGTACATAACGAAGCGTTCCCTACGACTTATGAAAGATGAATATGGCAATCAAAGTATGGTCGTTGATGAAGTCTTAC 15839
E. faecalis    GCGATACCGAAGCGATTAACCAGATTCTACATCATTACAGAGGGTACATAACGAAGCGTTCCCTACGACTTATGAAAGATGAATATGGCAATCAAAGTATGGTCGTTGATGAAGTCTTAC 15839
S. suis        GCGATACCGAAGCGATTAACCAGATTCTACATCATTACAGAGGGTACATAACGAAGCGTTCCCTACGACTTATGAAAGATGAATATGGCAATCAAAGTATGGTCGTTGATGAAGTCTTAC 15838
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S. parauberis   GTGGAAGAATGGAAACCAGACTGATTACAAAGATTTTGTCATTTGAAATTAAGTAATATCCTCTCTCCTTTCGTGGAAGCGTGCTAAACCATTCCACGCTTCCCGAACAGGGAGGTTTGT 15958
S. agalatiae    GTGGAAGAATGGAAACCAGACTGATTACAAAGATTTTGTCATTTGAAATTAAGTAATATCCTCTCTCCTTTCGTGGAAGCGTGCTAAACCATTCCACGCTTCCCGAACAGGGAGGTTTGT 15958
pCF10           GTGGAAGAATGGAAACCAGACTGATTACAAAGATTTTGTCATTTGAAATTAAGTAATATCCTCTCTCCTTTCGTGGAAGCGTGCTAAACCATTCCACGCTTCCCGAACAGGGAGGTTTGT 15959
E. faecalis     GTGGAAGAATGGAAACCAGACTGATTACAAAGATTTTGTCATTTGAAATTAAGTAATATCCTCTCTCCTTTCGTGGAAGCGTGCTAAACCATTCCACGCTTCCCGAACAGGGAGGTTTGT 15959
S. suis         GTGGAAGAATGGAAACCAGACTGATTACAAAGATTTTGTCATTTGAAATTAAGTAATATCCTCTCTCCTTTCGTGGAAGCGTGCTAAACCATTCCACGCTTCCCGAACAGGGAGGTTTGT 15958
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S. parauberis   TATTCCACCAAAGCATATTGAGCTTTCAATGTGTTTTGATAGGCTAACGAGCCATTGTTCTTTGAAAACTGAATAAAAGTAATCGAATACGTTTCGATAAGAAAAGAGCCAACGGAACTA 16078
S. agalatiae    TATTCCACCAAAGCATATTGAGCTTTCAATGTGTTTTGATAGGCTAACGAGCCATTGTTCTTTGAAAACTGAATAAAAGTAATCGAATACGTTTCGATAAGAAAAGAGCCAACGGAACTA 16078
pCF10           TATTCCACCAAAGCATATTGAGCTTTCAATGTGTTTTGATAGGCTAACGAGCCATTGTTCTTTGAAAACTGAATAAAAGTAATCGAATACGTTTCGATAAGAAAAGAGCCAACGGAACTA 16079
E. faecalis     TATTCCACCAAAGCATATTGAGCTTTCAATGTGTTTTGATAGGCTAACGAGCCATTGTTCTTTGAAAACTGAATAAAAGTAATCGAATACGTTTCGATAAGAAAAGAGCCAACGGAACTA 16079
S. suis         TATTCCACCAAAGCATATTGAGCTTTCAATGTGTTTTGATAGGCTAACGAGCCATTGTTCTTTGAAAACTGAATAAAAGTAATCGAATACGTTTCGATAAGAAAAGAGCCAACGGAACTA 16078
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S. parauberis   ACCGCCATGACCTATCTTATAAAGATAGCGAGCGATTCATGTTAGTGATCCGAGAAGCAATCTTTAGCAGGATTGCCTGCAACGACATTCTTATCGTGATAATGATACTCCCATACAGTC 16198
S. agalatiae    ACCGCCATGACCTATCTTATAAAGATAGCGAGCGATTCATGTTAGTGATCCGAGAAGCAATCTTTAGCAGGATTGCCTGCAACGACATTCTTATCGTGATAATGATACTCCCATACAGTC 16198
pCF10           ACCGCCATGACCTATCTTATAAAGATAGCGAGCGATTCATGTTAGTGATCCGAGAAGCAATCTTTAGCAGGATTGCCTGCAACGACATTCTTATCGTGATAATGATACTCCCATACAGTC 16199
E. faecalis     ACCGCCATGACCTATCTTATAAAGATAGCGAGCGATTCATGTTAGTGATCCGAGAAGCAATCTTTAGCAGGATTGCCTGCAACGACATTCTTATCGTGATAATGATACTCCCATACAGTC 16199
S. suis         ACCGCCATGACCTATCTTATAAAGATAGCGAGCGATTCATGTTAGTGATCCGAGAAGCAATCTTTAGCAGGATTGCCTGCAACGACATTCTTATCGTGATAATGATACTCCCATACAGTC 16198
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S. parauberis   AATAGTCCGAGCGTGATAAAACCGTCGCAGGCAATGAGTATGGCTACATGAGAACCATGCAGGGGTGGAACTCCCGTGAGCTTTGCTAAAGCTGTTCGATTGCTGGTAAAACAACTTTTA 16318
S. agalatiae    AATAGTCCGAGCGTGATAAAACCGTCGCAGGCAATGAGTATGGCTACATGAGAACCATGCAGGGGTGGAACTCCCGTGAGCTTTGCTAAAGCTGTTCGATTGCTGGTAAAACAACTTTTA 16318
pCF10           AATAGTCCGAGCGTGATAAAACCGTCGCAGGCAATGAGTATGGCTACATGAGAACCATGCAGGGGTGGAACTCCCGTGAGCTTTGCTAAAGCTGTTCGATTGCTGGTAAAACAACTTTTA 16319
E. faecalis     AATAGTCCGAGCGTGATAAAACCGTCGCAGGCAATGAGTATGGCTACATGAGAACCATGCAGGGGTGGAACTCCCGTGAGCTTTGCTAAAGCTGTTCGATTGCTGGTAAAACAACTTTTA 16319
S. suis         AATAGTCCGAGCGTGATAAAACCGTCGCAGGCAATGAGTATGGCTACATGAGAACCATGCAGGGGTGGAACTCCCGTGAGCTTTGCTAAAGCTGTTCGATTGCTGGTAAAACGACTTTTA 16318
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S. parauberis   TGAAATCCAAATAAGTGATTTGGAAAGGAGGATTTTATGAAGCAGACTGACATTCCTATTTGGGAACGTTATACCCTAACCATTGAAGAAGCGTCAAAATATTTTCGTATTGGCGAAAAC 16438
S. agalatiae    TGAAATCCAAATAAGTGATTTGGAAAGGAGGATTTTATGAAGCAGACTGACATTCCTATTTGGGAACGTTATACCCTAACCATTGAAGAAGCGTCAAAATATTTTCGTATTGGCGAAAAC 16438
pCF10           TGAAATCCAAATAAGTGATTTGGAAAGGAGGATTTTATGAAGCAGACTGACATTCCTATTTGGGAACGTTATACCCTAACCATTGAAGAAGCGTCAAAATATTTTCGTATTGGCGAAAAC 16439
E. faecalis     TGAAATCCAAATAAGTGATTTGGAAAGGAGGATTTTATGAAGCAGACTGACATTCCTATTTGGGAACGTTATACCCTAACCATTGAAGAAGCGTCAAAATATTTTCGTATTGGCGAAAAC 16439
S. suis         TGAAATCCAAATAAGTGATTTGGAAAGGAGGATTTTATGAAGCAGACTGACATTCCTATTTGGGAACGTTATACCCTAACCATTGAAGAAGCGTCAAAATATTTTCGTATTGGCGAAAAC 16438
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S. parauberis   AAGCTACGACGCTTGGCAGAGGAAAATAAAAATGCAAATTGGCTGATTATGAATGGCAATCGTATTCAGATTAAACGAAAACAATTTGAAAAAATTATAGATACATTGGACGCAATCTAG 16558
S. agalatiae    AAGCTACGACGCTTGGCAGAGGAAAATAAAAATGCAAATTGGCTGATTATGAATGGCAATCGTATTCAGATTAAACGAAAACAATTTGAAAAAATTATAGATACATTGGACGCAATCTAG 16558
pCF10           AAGCTACGACGCTTGGCAGAGGAAAATAAAAATGCAAATTGGCTGATTATGAATGGCAATCGTATTCAGATTAAACGAAAACAATTTGAAAAAATTATAGATACATTGGACGCAATCTAG 16559
E. faecalis     AAGCTACGACGCTTGGCAGAGGAAAATAAAAATGCAAATTGGCTGATTATGAATGGCAATCGTATTCAGATTAAACGAAAACAATTTGAAAAAATTATAGATACATTGGACGCAATCTAG 16559
S. suis         AAGCTACGACGCTTGGCAGAGGAAAATAAAAATGCAAATTGGCTGATTATGAATGGCAATCGTATTCAGATTAAACGAAAACAATTTGAAAAAATTATAGATACATTGGACGCAATCTAG 16558
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S. parauberis   CGTCGCCAAAGGGTCTTGTATATGATAAAATAGTATTAAGTCGTATCAAGGCTCTTTCCATAAAGGAAAGGAGCAAATGCCATGTCAGAAAAAAGACGTGACAATAAAGGTCGAATCTTA 16678
S. agalatiae    CGTCGCCAAAGGGTCTTGTATATGATAAAATAGTATTAAGTCGTATCAAGGCTCTTTCCATAAAGGAAAGGAGCAAATGCCATGTCAGAAAAAAGACGTGACAATAAAGGTCGAATCTTA 16678
pCF10           CGTCGCCAAAGGGTCTTGTATATGATAAAATAGTATTAAGTCGTATCAAGGCTCTTTCCATAAAGGAAAGGAGCAAATGCCATGTCAGAAAAAAGACGTGACAATAAAGGTCGAATCTTA 16679
E. faecalis     CGTCGCCAAAGGGTCTTGTATATGATAAAATAGTATTAAGTCGTATCAAGGCTCTTTCCATAAAGGAAAGGAGCAAATGCCATGTCAGAAAAAAGACGTGACAATAGAGGTCGAATCTTA 16679
S. suis         CGTCGCCAAAGGGTCTTGTATATGATAAAATAGTATTAAGTCGTATCAAGGCTCTTTCCATAAAGGAAAGGAGCAAATGCCATGTCAGAAAAAAGACGTGACAATAAAGGTCGAATCTTA 16678
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S. parauberis   AAGACTGGAGAGAGCCAACGAAAAGACGGAAGATACTTATACAAATATATAGATTCATTTGGAGAACCGCAATTTGTTTACTCGTGGAAACTTGTGGCTACAGACCGAGTACCAGCAGGA 16798
S. agalatiae    AAGACTGGAGAGAGCCAACGAAAAGACGGAAGATACTTATACAAATATATAGATTCATTTGGAGAACCGCAATTTGTTTACTCGTGGAAACTTGTGGCTACAGACCGAGTACCAGCAGGA 16798
pCF10           AAGACTGGAGAGAGCCAACGAAAAGACGGAAGATACTTATACAAATATATAGATTCATTTGGAGAACCGCAATTTGTTTACTCGTGGAAACTTGTGGCTACAGACCGAGTACCAGCAGGA 16799
E. faecalis     AAGACTGGAGAGAGCCAACGAAAAGACGGAAGATACTTATACAAATATATAGATTCATTTGGAGAACCGCAATTTGTTTACTCGTGGAAACTTGTGGCTACAGACCGAGTACCAGCAGGA 16799
S. suis         AAGACTGGAGAGAGCCAACGAAAAGACGGAAGATACTTATACAAATATATAGATTCATTTGGAGAACCGCAATTTGTTTACTCGTGGAAACTTGTGGCTACAGACCGAGTACCAGCAGGA 16798
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S. parauberis   AAGCGTGATTGTATCTCACTTAGAGAGAAAATCGCAGAGTTACAGAAAGACATTCATGATGGTATTGATGTTGTAGGAAAGAAAATGACACTCTGCCAGCTTTACGCAAAACAGAACGCT 16918
S. agalatiae    AAGCGTGATTGTATCTCACTTAGAGAGAAAATCGCAGAGTTACAGAAAGACATTCATGATGGTATTGATGTTGTAGGAAAGAAAATGACACTCTGCCAGCTTTACGCAAAACAGAACGCT 16918
pCF10           AAGCGTGATTGTATCTCACTTAGAGAGAAAATCGCAGAGTTACAGAAAGACATTCATGATGGTATTGATGTTGTAGGAAAGAAAATGACACTCTGCCAGCTTTACGCAAAACAGAACGCT 16919
E. faecalis     AAGCGTGATTGTATCTCACTTAGAGAGAAAATCGCAGAGTTACAGAAAGACATTCATGATGGTATTGATGTTGTAGGAAAGAAAATGACACTCTGCCAGCTTTACGCAAAACAGAACGCT 16919
S. suis         AAGCGTGATTGTATCTCACTTAGAGAGAAAATCGCAGAGTTACAGAAAGACATTCATGATGGTATTGATGTTGTAGGAAAGAAAATGACACTCTGCCAGCTTTACGCAAAACAGAACGCT 16918
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S. parauberis   CAAAGACCAAAGGTTAGAAAAAACACTGAAACTGGACGCAAATATCTTATGGATATTTTGAAGAAAGACAAGTTAGGTGTAAGAAGTATTGACAGTATTAAGCCATCAGACGCTAAAGAA 17038
S. agalatiae    CAAAGACCAAAGGTTAGAAAAAACACTGAAACTGGACGCAAATATCTTATGGATATTTTGAAGAAAGACAAGTTAGGTGTAAGAAGTATTGACAGTATTAAGCCATCAGACGCTAAAGAA 17038
pCF10           CAAAGACCAAAGGTTAGAAAAAACACTGAAACTGGACGCAAATATCTTATGGATATTTTGAAGAAAGACAAGTTAGGTGTAAGAAGTATTGACAGTATTAAGCCATCAGACGCTAAAGAA 17039
E. faecalis     CAAAGACCAAAGGTTAGAAAAAACACTGAAACTGGACGCAAATATCTTATGGATATTTTGAAGAAAGACAAGTTAGGTGTAAGAAGTATTGACAGTATTAAGCCATCAGACGCTAAAGAA 17039
S. suis         CAAAGACCAAAGGTTAGAAAAAACACTGAAACTGGACGCAAATATCTTATGGATATTTTGAAGAAAGACAAGTTAGGTGTAAGAAGTATTGACAGTATTAAGCCATCAGACGCTAAAGAA 17038
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S. parauberis   TGGGCTATTAGAATGAGTGAAAATGGTTATGCTTATCAAACCATCAATAACTACAAACGTTCTTTAAAGGCTTCATTCTATATTGCTATACAAGATGATTGTGTTCGGAAGAATCCATTT 17158
S. agalatiae    TGGGCTATTAGAATGAGTGAAAATGGTTATGCTTATCAAACCATCAATAACTACAAACGTTCTTTAAAGGCTTCATTCTATATTGCTATACAAGATGATTGTGTTCGGAAGAATCCATTT 17158
pCF10           TGGGCTATTAGAATGAGTGAAAATGGTTATGCTTATCAAACCATCAATAACTACAAACGTTCTTTAAAGGCTTCATTCTATATTGCTATACAAGATGATTGTGTTCGGAAGAATCCATTT 17159
E. faecalis     TGGGCTATTAGAATGAGTGAAAATGGTTATGCTTATCAAACCATCAATAACTACAAACGTTCTTTAAAGGCTTCATTCTATATTGCTATACAAGATGATTGTGTTCGGAAGAATCCATTT 17159
S. suis         TGGGCTATTAGAATGAGTGAAAATGGTTATGCTTATCAAACCATCAATAACTACAAACGTTCTTTAAAGGCTTCATTCTATATTGCTATACAAGATGATTGTGTTCGGAAGAATCCATTT 17158
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S. parauberis    GACTTTCAACTGAAAGCAGTTCTTGATGATGATACTGTCCCTAAGACCGTACTAACAGAAGAACAGGAAGAAAAACTGTTAGCCTTTGCAAAAGCTGATAAAACCTACAGCAAAAATTAT 17278
S. agalatiae     GACTTTCAACTGAAAGCAGTTCTTGATGATGATACTGTCCCTAAGACCGTACTAACAGAAGAACAGGAAGAAAAACTGTTAGCCTTTGCAAAAGCTGATAAAACCTACAGCAAAAATTAT 17278
pCF10            GACTTTCAACTGAAAGCAGTTCTTGATGATGATACTGTCCCTAAGACCGTACTAACAGAAGAACAGGAAGAAAAACTGTTAGCCTTTGCAAAAGCTGATAAAACCTACAGCAAAAATTAT 17279
E. faecalis      GACTTTCAACTGAAAGCAGTTCTTGATGATGATACTGTCCCTAAGACCGTACTAACAGAAGAACAGGAAGAAAAACTGTTAGCCTTTGCAAAAGCTGATAAAACCTACAGCAAAAATTAT 17279
S. suis          GACTTTCAACTGAAAGCAGTTCTTGATGATGATACTGTCCCTAAGACCGTACTAACAGAAGAACAGGAAGAAAAACTGTTAGCCTTTGCAAAAGCTGATAAAACCTACAGCAAAAATTAT 17278
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S. parauberis    GATGAAATTCTGATACTCTTAAAAACAGGTCTTCGTATTTCAGAGTTTGGTGGTTTGACACTTCCAGATTTAGATTTTGAGAATCGTCTTGTCAATATAGACCATCAGCTATTGAGAGAT 17398
S. agalatiae     GATGAAATTCTGATACTCTTAAAAACAGGTCTTCGTATTTCAGAGTTTGGTGGTTTGACACTTCCAGATTTAGATTTTGAGAATCGTCTTGTCAATATAGACCATCAGCTATTGAGAGAT 17398
pCF10            GATGAAATTCTGATACTCTTAAAAACAGGTCTTCGTATTTCAGAGTTTGGTGGTTTGACACTTCCAGATTTAGATTTTGAGAATCGTCTTGTCAATATAGACCATCAGCTATTGAGAGAT 17399
E. faecalis      GATGAAATTCTGATACTCTTAAAAACAGGTCTTCGTATTTCAGAGTTTGGTGGTTTGACACTTCCAGATTTAGATTTTGAGAATCGTCTTGTCAATATAGACCATCAGCTATTGAGAGAT 17399
S. suis          GATGAAATTCTGATACTCTTAAAAACAGGTCTTCGTATTTCAGAGTTTGGTGGTTTGACACTTCCAGATTTAGATTTTGAGAATCGTCTTGCCAATATAGACCATCAGCTATTGAGAGAT 17398
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S. parauberis    ACTGAAATTGGGTACTACATTGAAACACCAAAGACCAAAAGTGGCGAACGTCAAGTTCCTATGGTTGAAGAAGCCTATCAAGCATTTAAGCGAGTGTTAGCGAATCGAAAGAATGATAAG 17518
S. agalatiae     ACTGAAATTGGGTACTACATTGAAACACCAAAGACCAAAAGTGGCGAACGTCAAGTTCCTATGGTTGAAGAAGCCTATCAAGCATTTAAGCGAGTGTTAGCGAATCGAAAGAATGATAAG 17518
pCF10            ACTGAAATTGGGTACTACATTGAAACACCAAAGACCAAAAGTGGCGAACGTCAAGTTCCTATGGTTGAAGAAGCCTATCAAGCATTTAAGCGAGTGTTAGCGAATCGAAAGAATGATAAG 17519
E. faecalis      ACTGAAATTGGGTACTACATTGAAACACCAAAGACCAAAAGTGGCGAACGTCAAGTTCCTATGGTTGAAGAAGCCTATCAAGCATTTAAGCGAGTGTTAGCGAATCGAAAGAATGATAAG 17519
S. suis          ACTGAAATTGGGTACTACATTGAAACACCAAAGACCAAAAGTGGCGAACGTCAAGTTCCTATGGTTGAAGAAGCCTATCAAGCATTTAAGCGAGTGTTAGCGAATCGAAAGAATGATAAG 17518
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S. parauberis    CGTGTTGAGATTGATGGATATAGTGATTTCCTCTTTCTTAATAGAAAGAACTATCCAAAAGTGGCAAGTGATTACAACGGCATGATGAAAGGTCTTGTTAAGAAATACAATAAGTATAAC 17638
S. agalatiae     CGTGTTGAGATTGATGGATATAGTGATTTCCTCTTTCTTAATAGAAAGAACTATCCAAAAGTGGCAAGTGATTACAACGGCATGATGAAAGGTCTTGTTAAGAAATACAATAAGTATAAC 17638
pCF10            CGTGTTGAGATTGATGGATATAGTGATTTCCTCTTTCTTAATAGAAAGAACTATCCAAAAGTGGCAAGTGATTACAACGGCATGATGAAAGGTCTTGTTAAGAAATACAATAAGTATAAC 17639
E. faecalis      CGTGTTGAGATTGATGGATATAGTGATTTCCTCTTTCTTAATAGAAAGAACTATCCAAAAGTGGCAAGTGATTACAACGGCATGATGAAAGGTCTTGTTAAGAAATACAATAAGTATAAC 17639
S. suis          CGTGTTGAGATTGATGGATATAGTGATTTCCTCTTTCTTAATAGAAAGAACTATCCAAAAGTGGCAAGTGATTACAACGGCATGATGAAAGGTCTTGTTAAGAAATACAATAAGTATAAC 17638
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S. parauberis    GAGGATAAATTGCCACACATCACTCCACATAGTTTGCGACATACATTCTGTACCAACTATGCAAATGCAGGAATGAATCCAAAGGCATTACAGTACATTATGGGACATGCTAATATAGCC 17758
S. agalatiae     GAGGATAAATTGCCACACATCACTCCACATAGTTTGCGACATACATTCTGTACCAACTATGCAAATGCAGGAATGAATCCAAAGGCATTACAGTACATTATGGGACATGCTAATATAGCC 17758
pCF10            GAGGATAAATTGCCACACATCACTCCACATAGTTTGCGACATACATTCTGTACCAACTATGCAAATGCAGGAATGAATCCAAAGGCATTACAGTACATTATGGGACATGCTAATATAGCC 17759
E. faecalis      GAGGATAAATTGCCACACATCACTCCACATAGTTTGCGACATACATTCTGTACCAACTATGCAAATGCAGGAATGAATCCAAAGGCATTACAGTACATTATGGGACATGCTAATATAGCC 17759
S. suis          GAGGATAAATTGCCACACATCACTCCACATAGTTTGCGACATACATTCTGTACCAACTATGCAAATGCAGGAATGAATCCAAAGGCATTACAGTACATTATGGGACATGCTAATATAGCC 17758
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S. parauberis    ATGACGCTGAACTATTACGCACATGCAACATTCGATTCTGCAATGGCAGAAATGAAACGCTTGAATAAAGAGAAGCAACAGGAGCGTCTTGTTGCTTAGTAGTACAAATGAATTTACTAC 17878
S. agalatiae     ATGACGCTGAACTATTACGCACATGCAACATTCGATTCTGCAATGGCAGAAATGAAACGCTTGAATAAAGAGAAGCAACAGGAGCGTCTTGTTGCTTAGTAGTACAAATGAATTTACTAC 17878
pCF10            ATGACGCTGAACTATTACGCACATGCAACATTCGATTCTGCAATGGCAGAAATGAAACGCTTGAATAAAGAGAAGCAACAGGAGCGTCTTGTTGCTTAGTAGTACAAATGAATTTACTAC 17879
E. faecalis      ATGACGCTGAACTATTACGCACATGCAACATTCGATTCTGCAATGGCAGAAATGAAACGCTTGAATAAAGAGAAGCAACAGGAGCGTCTTGTTGCTTAGTAGTACAAATGAATTTACTAC 17879
S. suis          ATGACGCTGAACTATTACGCACATGCAACATTCGATTCTGCAATGGCAGAAATGAAACGCTTGAGTAAAGAGAAGCAACAGGAGCGTCTTGTTGCTTAGTAGTACAAATGAATTTACTAC 17878
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S. parauberis    TTATTTACCACTTCTGACAGCTAAGACATGAGGAAATATGCAAAGAAACGTGAAGTATCTTCCTACAGTAAAAATACTCGAAAGCACATAGAATAAGGCTTTACGAGCATTTAAGAAAAT 17998
S. agalatiae     TTATTTACCACTTCTGACAGCTAAGACATGAGGAAATATGCAAAGAAACGTGAAGTATCTTCCTACAGTAAAAATACTCGAAAGCACATAGAATAAGGCTTTACGAGCATTTAAGAAAAT 17998
pCF10            TTATTTACCACTTCTGACAGCTAAGACATGAGGAAATATGCAAAGAAACGTGAAGTATCTTCCTACAGTAAAAATACTCGAAAGCACATAGAATAAGGCTTTACGAGCATTTAAGAAAAT 17999
E. faecalis      TTATTTACCACTTCTGACAGCTAAGACATGAGGAAATATGCAAAGAAACGTGAAGTATCTTCCTACAGTAAAAATACTCGAAAGCACATAGAATAAGGCTTTACGAGCATTTAAGAAAAT 17999
S. suis          TTATTTACCACTTCTGACAGCTAAGACATGAGGAAATATGCAAAGAAACGTGAAGTATCTTCCTACAGTAAAAATACTCGAAAGCACATAGAATAAGGCTTTACGAGCATTTAAGAAAAT 17998
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S. parauberis    ATAAAAAGATAATTAGAAATTTATACTTTGTTT- 18031
S. agalatiae     ATAAAAAGATAATTAGAAATTTATACTTTGTTTT 18032
pCF10            ATAAAAAGATAATTAGAAATTTATACTTTGTTTA 18033
E. faecalis      ATAAAAAGATAATTAGAAATTTATACTTTGTTT- 18032
S. suis          ATAAAAAGATAATTAGAAATTTATACTTTGTTT- 18031
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Fig. 4-3. Alignment of the nucleotide sequences of Tn*916* from *S. parauberis* NUF1049 (accession number AB468159) with those of *S. agalactiae* 2603V/R Tn*916*-like element (accession number AE009948), *E. faecalis* plasmid pCF10 Tn*925* (accession number AY855841), E. *faecalis* DS16 Tn*916* (accession number U09422), and *S. suis* SC84 Tn*916* (accession number EF432727) generated using the ClustalW software. The asterisk (*) shows sequences that are the same as those of Tn*916* sequence. The organism is given on the left of each sequence lane. The sequence number is given on the right of each sequence lane.

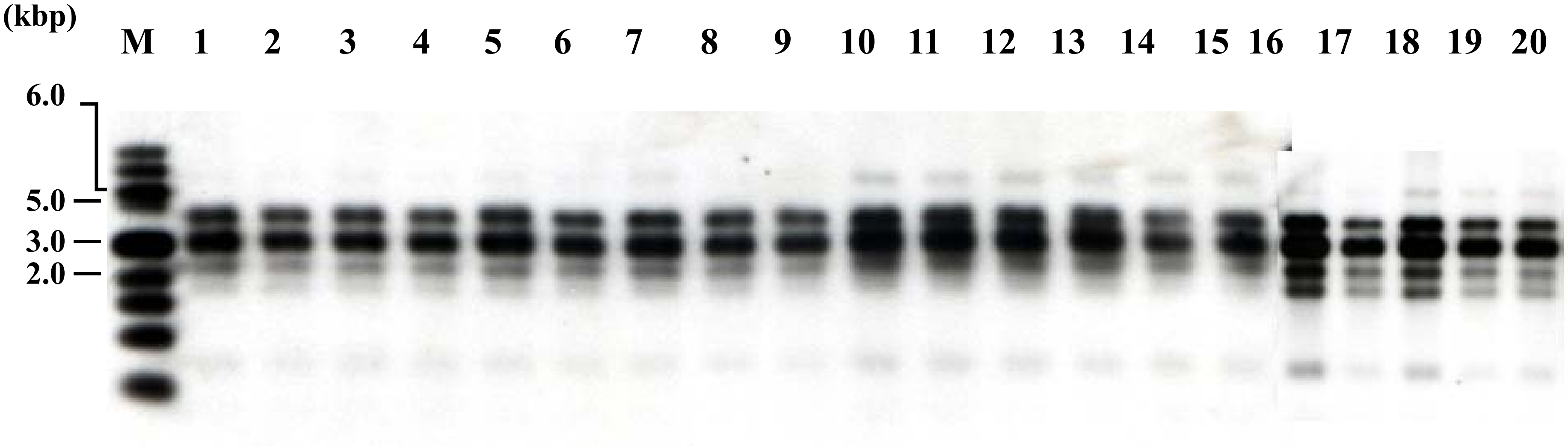

Fig. 4-4. Southern hybridization analysis of Tn*916*-like element. Chromosomal DNAs were digested with *Sau*3AI. The digests on the membrane were hybridized with DIG-labeled Tn*916*-like element probe. Lane M: *E. faecalis* CG110 (positive control); Lane 1-20: *S. parauberis* serotype II strains tested in this study.

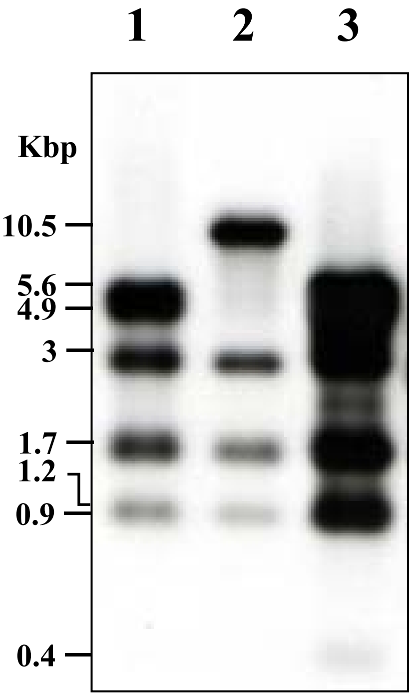

Fig. 4-5. Southern blot hybridization analysis of Tn*916*-like element. Chromosomal DNAs were digested with *Hin*cII. The digests on the membrane were hybridized with DIG-labeled Tn*916*-like element probe. Lane 1, Pattern I of serotype II strain; Lane 2, Pattern II of serotype II strain ; Lane 3, *E. faecalis* CG110 (positive control of Tn*916*).

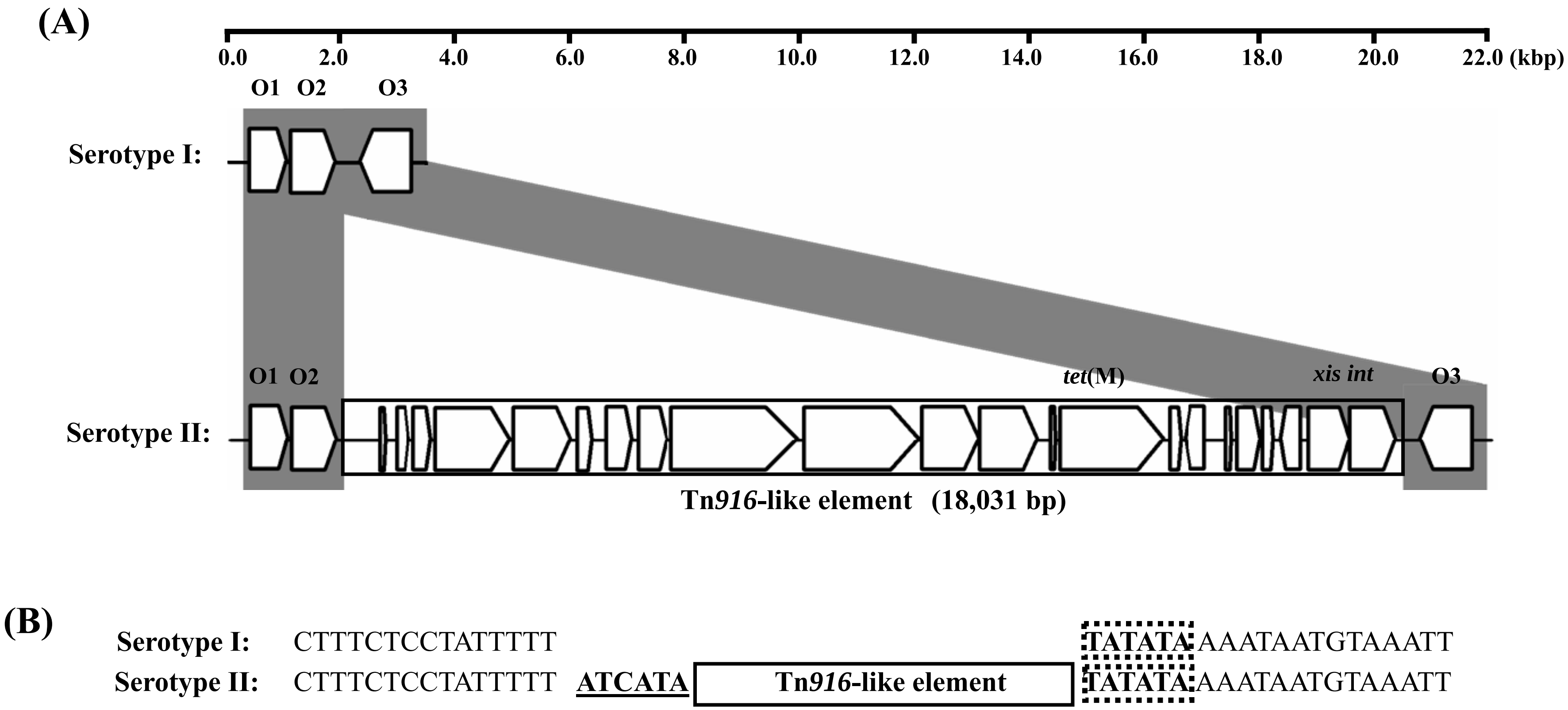

Fig. 4-6. (A)Structural analysis of *S. parauberis* serotype II strains (NUF1049) and *S. parauberis* serotype I strains (NUF1003) in the areas of the Tn*916*-like element ORF and the related flanking regions ORF (white arrow). The gray area indicates homology areas of *S. parauberis*. (B) Flanking sequences of the Tn*916*-like element in *S. parauberis*. Coupling sequences are indicated by boldface and underline.

General discussion

At the beginning of 21 century, sporadic streptococcal infections were identified in Kagawa, Ehime, Oita and Nagasaki Prefectures. The disease signs differed from those of *S. iniae* infection. The causative agent of the new outbreaks was identified as *S. parauberis*. Currently, the spread of *S. parauberis* has been across the western districts of Japan and emerged as a leading cause of streptococcal infection in Japanese flounder.

In this study, two points have been first reported. One is that *S. parauberis* were divided into two serotypes according to serological differentiations. The other one is that all of the serotype II strains (used in this study) harbored Tn*916*-like element with *tet*(M) resistance gene.

Serotyping of bacteria has been widely used as a tool in bacterial epidemiology. Based on the serological investigation in this study, the structurally and immunologically different capsular polysaccharides exist in the Japanese isolates of *S. parauberis*. So with the rabbit antisera reacting specifically with *S. parauberis* serotype I and II, serological methods such as slide agglutination could be conveniently used for clinical diagnosis of *S. parauberis* infections in Japanese flounder.

Comparison of MIC values of *S. parauberis* susceptible strains with those of *S. iniae* not only showed similar MIC values but also exhibit an intrinsic resistance to OA and SMMX. Unfortunately, the resistant determinants for OTC, that is the only qualified drug in treating Japanese flounder streptococcal infection, have spread among *S. parauberis* in different sites. But in our study the effectiveness of the common drugs, such as ABPC and EM, were proved for *S. parauberis* infection therapy.

The worldwide use of tetracyclines in the treatment of infections, as prophylactic agents, and as growth promoters, has facilitated the emergence and spread of acquired resistance (Roberts, 2005). Furthermore, resistant bacteria may rapidly appear in the host or environment after antimicrobial use, but they are slow to be lost, even in the absence of the selecting antimicrobial (Levy and Marshall, 2004). The PCR and hybridization results implied that acquisitions of OTC resistance in *S. parauberis* occur through different mechanisms. *S. parauberis* serotype I strains were detected highly resistant to OTC mediated by a conjugative R-plasmid encoding *tet*(S), while all the serotype II strains were intermediately resistant mediated by the Tn*916*-like element harboring *tet*(M). The data support the contention that Tn*916*-like element with *tet*(M) acts as an important selective factor that provides considerable advantages for the emergence and spread of *S. parauberis* serotype II.

Five EM/OTC-resistant serotype I and six serotype II strains, chosen as representative of the different prefectures, was used as donors in mating experiments. *tet*(S) was the sole that transferred to the recipient from all of the *tet*(S)-positive serotype I donors (only when OTC was used for selection), suggesting no linkage of the two resistance genes [*erm*(B) and *tet*(S)] in different genetic elements. Likewise, hybridization experiments indicated that the chromosomal band hybridized only with an *erm*(B)-specific probe. The variabilities of the resistant genes insertions suggested the involvement of a number of different genetic elements carrying *tet*(S) and *erm*(B), respectively, in serotype I strains. In conjugative transfer experiment no transconjugants were obtained when six *S. parauberis* serotype II strains from different prefectures were used as donors and *E. faecalis* FA2-2 as the recipient. Transfer frequency was calculated to be less than 10^{-9}.

Based on the cloning and sequencing the *erm*(B) gene containing region in serotype I resistant strains, it was found to show high homology with other known *erm*(B) gene containing regions in plasmids. Moreover, the important structural feature found conserved in the relative regions was present in serotype I resistant strains.

The present study demonstrated that the Tn*916*-like element that exhibited high structural homology with Tn*916* or Tn*916*-like transposons from the other streptococci and enterococci were observed in all of examined *S. parauberis* serotype II strains isolated from diseased Japanese flounder. Restriction endonuclease analysis suggested the presence of some diversity in the nucleotide sequence within the strains, but the Tn*916*-like element was integrated in the same site of chromosome for all of the strains. To our knowledge it is the first case that all the strains of a particular group of bacterial species harbored a Tn*916* family transposon.

Tn*916* family conjugative transposons have been found in a broad variety of Gram-positive and Gram-negative organisms and become important vehicles for disseminating antimicrobial resistance through cell to cell contact (Rice 1998). In this study, however, horizontal transfer of the Tn*916*-like element was failed. Conjugative transposition of Tn*916* is via an excision-insertion mechanism with three stages; excision, conjugal transfer and integration (Caparon *et al.* 1989). Excision begins with generating single strands of 6-bp termed coupling squences flanking the transposon in the donor DNA (Scott, 1992). The nucleotide content of the coupling sequences plays a frequency-determining role upon excision of Tn*916* with unknown mechanism (Jaworski and Clewell, 1994). Therefore, conjugative transposition of Tn*916* occurred at widely differing frequencies ranging from less than 10^{-8} to greater than 10^{-4} per donor depending on the coupling sequences (Jaworski and Clewell, 1994). In this study, the sequencing results of the chromosomal region where the Tn*916*-like element was integrated provided evidence that the Tn*916*-like element introduced a coupling sequence (ATCATA) from its previous host. Accordingly, the 6-bp sequences adjacent to the Tn*916*-like element (ATCATA and TATATA) may result in its extreme low transfer frequency.

Although serotypes were not documented, a high rate of tetracycline resistance due to *tet*(M) was also reported in Korean isolates of *S. parauberis* (Park *et al.* in press). According to our findings it is likely that the prevalence of the *tet*(M)-positive serotype II strains in Japan are due to clonal dissemination of a original strain that acquired the Tn*916*-like element from another bacterial species rather than selection of *tet*(M)-positive strains by frequent use of tetracyclines. This hypothesis is supported by the fact that all of the tested serotype II strains were also low resistant to erythromycin in chapter I. It is thinkable that excision of the Tn*916*-like element from these strains is an extremely rare event resulting in all of the serotype II isolates possessing the element.

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要旨

ヒラメ由来*Streptococcus parauberis*における薬剤耐性菌の出現と耐性化機構に関する研究

Streptococcus parauberis はウシの乳房炎の原因菌として知られ，魚類ではターボット *Scophthalmus maximus* のレンサ球菌症の原因菌としてヨーロッパではじめて報告された。ヨーロッパ以外では，韓国において眼球突出と鰓の壊死を病徴とするヒラメ *Paralichthys olivaceus* の *S. parauberis* 感染症が報告されている。わが国では2000年代に入ってヒラメの *S. parauberis* 感染症の発生が確認され，その後発生地域が拡大して発生率および被害率が高い傾向が各地で見られている。

現在，ヒラメのレンサ球菌症の治療薬としてはテトラサイクリン系の抗生物質のみが承認されているが， *S. parauberis* の薬剤感受性および耐性菌の出現についての報告はない。本研究では，日本各地で分離された *S. parauberis* 株について，代表株で作製したウサギ抗血清との凝集性およびテトラサイクリンを含む主要な抗菌剤に対する感受性を調べた。そして薬剤耐性株については，耐性化機構について分子生物学的検討を行った。

（第一章）2002年から2007年にかけて西日本各地のヒラメ養殖場で分離された *S. parauberis* 64株は,すべてⅠ型（44株）あるいはⅡ型（20株）に分類された。血清型別した 64 株を供試菌株として，アンピシリン（ABPC)，カナマイシン（KM)，エリスロマイシン（EM)，リンコマイシン（LCM)，塩酸オキシテトラサイクリン（OTC)，クロラムフェニコール（CP)，オキソリン酸（OA)，スルファモノメトキシン（SMMX)，トリメトプリム（TMP）の 9 種類の抗菌剤の最小発育阻止濃度（MIC）を測定した。その結果，OAおよびSMMXについては高いMIC値を示したことから，*S. parauberis*が本来両薬剤に耐性であると考えられた。また，血清型がⅠ型の44株中5株がOTCとEMに高度耐性で，Ⅱ型は20株すべてがOTC中等度耐性を示した。

（第二章）見つかったOTCおよびEM耐性株から，耐性遺伝子および耐性遺伝子をコードするトランスポゾンに関連する遺伝子の検出を試みた。その結果，Ⅰ

型耐性株5株からはOTC耐性遺伝子*tet*(S)とEM耐性遺伝子*erm*(B)が検出され，Ⅱ型20株からはOTC耐性遺伝子 *tet*(M)およびトランスポゾン916（Tn*916*）の挿入酵素遺伝子 *int* と切出し酵素遺伝子 *xis* が検出された。このことから，Ⅱ型株にはTn*916*様の配列の存在が示唆された。

（第三章）血清型Ⅰ型株から検出された耐性遺伝子のゲノム上の位置をサザンハイブリダイゼーションで調べた。OTC/EM耐性株からは約11 kbpのプラスミドが検出され，各耐性遺伝子をプローブとしたサザンハイブリダイゼーションの結果，*tet*(S)はプラスミド上に，*erm*(B)は染色体DNA上にコードされていることが判明した。Ⅰ型耐性株5株に検出されたプラスミドはすべて受容菌*Enterococcus faecalis* FA2-2に伝達され，*Hin*dⅢによる切断パターンは同じであった。このことから，プラスミドは伝達性であり，同じプラスミドであると考えられた。染色体DNA上の*erm*(B)を含む*Hin*dⅢ断片をクローニングし，塩基配列を調べたところ，この断片は*erm*(B)の上流と下流に331 bpのリピート配列を有し，４つのORFからなる断片であり，その塩基配列は他の球菌のプラスミドの配列と92%以上の相同性を示した。したがって，この*erm*(B)を含む配列はプラスミド由来と推察された。

（第四章）血清型Ⅱ型株にTn*916*様のトランスポゾンの存在が示唆されたことから，GenBankから取得したTn*916*の塩基配列に基づいて設計したプライマーを用いて，Ⅱ型株の染色体DNAを鋳型にTn*916*の4つの部分に相当する配列のPCRを試みた。その結果，予想される長さの増幅産物がすべてのⅡ型株から得られ，本Tn*916*様配列がTn*916*と極めて類似する遺伝子構造を有することが推察された。代表株について得られたPCR産物の塩基配列を調べたところ，他の細菌のTn*916*とほぼ同じ配列であった。なお，Tn*916*様配列をプローブとしてサザンハイブリダイゼーションを行ったところ、*Sau*3AIおよび*Hin*dⅢの切断ハイブリダイゼーションパターンはⅡ型株すべて同一であったが，*Hinc*Ⅱの切断パターンは2種類認められた。したがって，*S. parauberis*Ⅱ型株由来のTn*916*様配列には配列に多様性があると考えられた。また，Tn*916*様配列に隣接する塩基配列を調べたところ，Tn*916*様配列の挿入部位はA/Tリッチな場所であり，Tn*916*様配

列を持たないⅠ型株の同じ部位の塩基配列との比較から，Tn*916*様配列にはATCATAの配列が付加されていることが判明した。

本研究から，*S. parauberis*血清型Ⅰ型株には伝達性プラスミドにコードされたOTC耐性遺伝子が，Ⅱ型株にはトランスポソンに保持されたOTC耐性遺伝子があることが明らかになった。ヒラメのレンサ球菌症ではオキシテトラサイクリン系薬剤が唯一使用できる治療薬であることから，耐性株の存在は*S. parauberis*感染症の防除対策を考える上で重要な問題である。Ⅰ型株ではプラスミドによって耐性が広まる懸念があり，Ⅱ型株ではすべての株が耐性である可能性があることから治療は困難である。今後はテトラサイクリンに代わる治療薬の開発あるいはワクチン等の予防法の確立を目指すことが必要と思われるが，耐性化を助長しないよう，ヒラメ養殖におけるテトラサイクリン系薬剤の使用は慎重に行わなければならない。