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Phylogenetic Analysis of the *Streptococcus agalactiae Sip* Gene in China

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Abstract: A *Streptococcus agalactiae* GS was isolated from milk from mastitis cattle in Gansu region of China. Sequence and phylogenetic analysis of *Sip* gene segments revealed that the *Streptococcus agalactiae* GS was most similar to a recent group B streptococcus isolated in dairy cattle from China. The *Sip* gene were selected and amplified, it contained a 1305 bp Open Reading Frame (ORF) which encoded 434 amino acids. The molecular mass of the deduced amino acid sequences was 56 kD. Blast analysis showed that it shared high identities (91.0-99.0%) with Sip sequences of other GBS registered in GenBank while lower identities (<50%) was found compared with other species of *Streptococcus*. The deduced amino acid homology was 90.2-98.8%.

Key words: *Streptococcus agalactiae*, Sip, phylogenetic, amino acid, China

INTRODUCTION

Streptococcus agalactiae (also known as group B Streptococcus or GBS) is a beta-hemolytic Gram-positive streptococcus. *S. agalactiae* was recognised as a pathogen in cattle before the Second World War. Its significance as human pathogen was not discovered before the 1950s. In cattle it causes mastitis, an infection of the udder. It can either give acute, febrile disease or sub-acute, more chronic disease. Both lead to diminishing milk production (hence its name: Agalactiae meaning "no milk"). Outbreaks in herds are common. This is of major significance for the dairy industry and programs to reduce the impact of *S. agalactiae* disease have been enforced in many countries over at least the last 30-40 years (Yildirim *et al*., 2002). It is also a pathogen of newborn infants in particular but also a cause of bacteremia in parturient women, elderly people and immune compromised patients. In aquaculture *S. agalactiae* is an emerging pathogen that has been associated with considerable morbidity and mortality in fish farms worldwide (Robinson and Meyer, 1966; Eldar *et al*., 1995; Duremdez *et al*., 2004; Mian *et al*., 2009). Occasionally, *S. agalactiae* has been associated with illness in many others hosts such as chickens, camels, dogs, horses, cats, frogs, hamsters, mice, monkeys, emerald monitors and nutria (Elliott *et al*., 1990; Yildirim *et al*., 2002; Hetzel *et al*., 2003; Johri *et al*., 2006).

The genetic diversity of *S. agalactiae* from human and bovine origins has been analyzed using a broad range of genomic techniques including ribotyping, RAPD, PFGE and MLST (Baseggio *et al*., 1997; Bisharat *et al*., 2004; Duarte *et al*., 2004; Oliveira *et al*., 2006; Honsa *et al*., 2008; Van Der Mee-Marquet *et al*., 2006). Previous studies of genetic relationships concluded that human and bovine strains belong to genetically distinct populations with limited interspecies transmission (Dogan *et al*., 2005; Sukhnanand *et al*., 2005) or common origin (Martinez *et al*., 2000). However, some reports described isolates of bovine and human origins that had the same genetic pattern (Bisharat *et al*., 2004; Bohnsack *et al*., 2004; Oliveira *et al*., 2006). Furthermore, the potential for *S. agalactiae* to cross the interspecies barrier and cause disease in others host such as fish remains unclear.

Surface immunogenic protein (Sip) is a kind of immune related protein that exists in group B Streptococcus which expresses in many serotypes of GBS. In this study, *Sip* gene was amplified by PCR of a bovine *S. agalactiae* strain isolated from milk, the Bioinformatics Software was used to analyze variety of the genetics.

The *Sip* gene contained a 1305 bp Open Reading Frame (ORF) which encoded 434 amino acids. The molecular mass of the deduced amino acid sequences was 56 kD. Blast analysis showed that it shared high identities (100%) with Sip sequences of human GBS registered in GenBank while lower identities (<50%) was found compared with other species of streptococcus. Prediction of epitopes by DNAStar Software showed that the deduced amino acid sequence of this *Sip* gene could form 29 epitopes indicating strong immunogenicity of the sip.

MATERIALS AND METHODS

Strains and vectors: The *S. agalactiae* strain was isolated from clinical mastitis of cow milk samples from Gansu

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Provence, China. *E. coli* DH5α was purchased from TaKaRa, pGEM-T Easy vector was purchased from Promega LTC.

Isolation and identification of bacteria: For isolation of *Streptococcus* sp., 0.5 mL of pooled milk sample was enriched in 5 mL of streptococcal selection broth (HiMedia Laboratories, Mumbai, India) and incubated at 37°C for 6 h with 5% CO_2. After enrichment, the samples were streaked onto 5% sheep blood agar and incubated at 37°C for 24-48 h with 5% CO_2. The suspected streptococcal colonies were purified on BHI agar (BD Biosciences, USA). The purified cultures were tentatively identified based on Gram's staining and biochemical tests namely, catalase, oxidase and bile esculin hydrolysis. To differentiate the suspected *S. uberis* isolates from enterococci, a selective media, KF streptococcal agar (BD Biosciences, USA) was also used.

DNA extraction: Bacterial DNA was extracted using QIAmp DNA mini kit (Qiagen, Duesseldorf, Germany) as per manufacturer's instructions. The concentration of the purified DNA was determined using NanoDrop 2000 (Thermo Fischer Scientific Inc, USA) and stored at -20°C until further use.

PCR and sequencing: PCRs were performed with primer SipF: 5'-ATGAAAATGAATAAAAAGGTAC-3' and SipR: 5'-TTATTTGTTAAATGATACGTG-3'. The target products were 1305 bp. Primers were synthesized by Invitrogen Trading Shanghai Co., Ltd. PCR reactions were cycled 35 times (94°C for 60 sec; 55°C for 45 sec; 72°C for 90 sec) with one final cycle at 72°C for 10 min in a thermocycler (Applied Biosystems 9700). PCR product was purified and constructed to pMD-18T vector. Sequencing of this PCR product allowed us to determine the sequence of the 5' and 3' ends of the gene as well as of one of the repetitions.

Phylogenetic analysis: In this study, phylogenetic trees for the *S. agalactiae Sip* gene and those of other 29 *S. agalactiae* strains were generated. The analysis was based on the nucleotide sequences of the *Sip* gene. The trees were generated with the MEGA Program (Version 5.0) by using neighbor-joining analysis.

RESULTS AND DISCUSSION

Isolation and identification of bacteria: Gram's staining and biochemical tests were used to confirm the purified cultures. A selective media, KF streptococcal agar (BD Biosciences, USA) was also used. The cultures were identified as *S. agalactiae*.

PCR amplification results: As expected, primers SipF/SipR yielded the expected PCR fragments. The PCR products were identified by electrophoresis and a fragment approximately 1305 bp were obtained (Fig. 1).

Phylogenetic analysis: Phylogenetic trees for the *S. agalactiae* isolates and other *S. agalactiae* strains were generated based on the nucleotide sequences of the *Sip* gene (Fig. 2). Phylogenetic analysis of *Sip* gene segments revealed that the *Streptococcus agalactiae* GS was most similar to a recent group B streptococcus isolated in dairy cattle from China. The BLAST results showed that it shared high identities (91.0-99.0%) with Sip sequences of other GBS registered in GenBank.

S. agalactiae is not only a major cause mastitis pathogens but also a serious threaten to tilapia and neonatal sepsis in humans. Understand their main antigens variation was very important.

In this study, researchers present the study that phylogenetic trees of this pathogen isolated from cows. The results clearly demonstrate that the *S. agalactiae* GS was most similar to a recent group B streptococcus isolated in dairy cattle from China. The *Sip* gene was

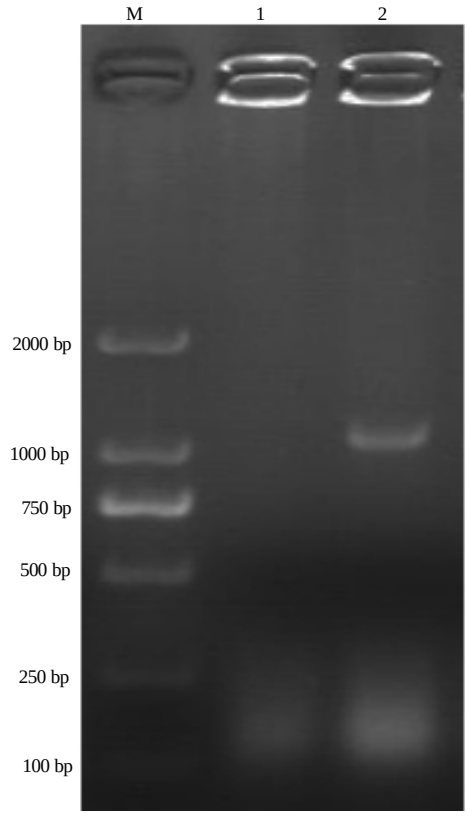

Fig. 1: PCR based on *Sip* gene. M: DL2000 Marker; Lane 1: Negative control; Lane 2: Sip fragments

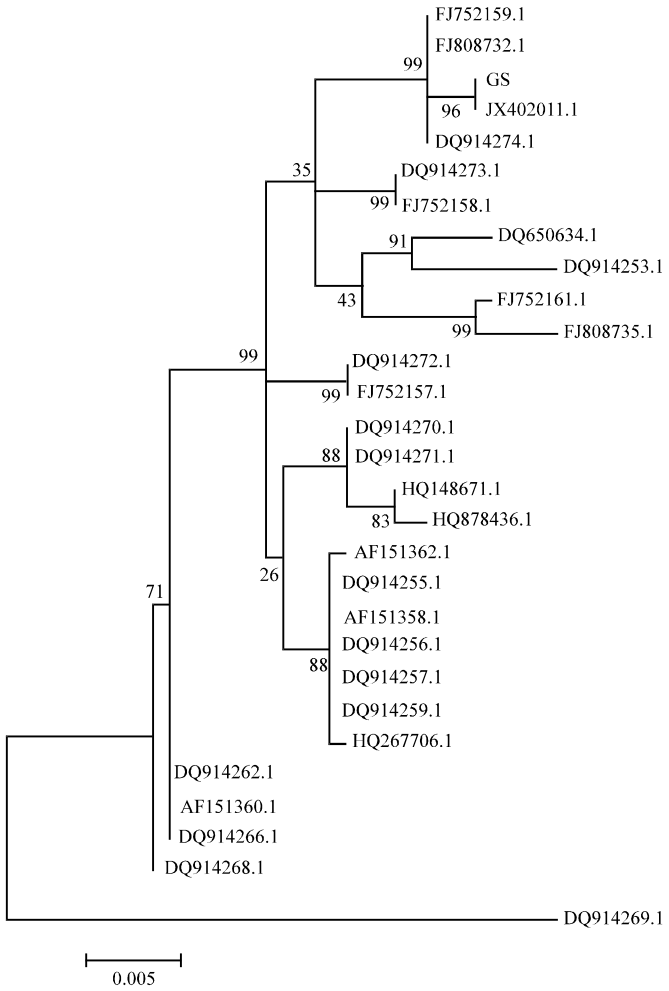

Fig. 2: Phylogenetic trees for the *S. agalactiae Sip* gene and those of other 29 *S. agalactiae* strains. The trees were generated with the MEGA Program (Version 5.0) by using neighbor-joining analysis

selected and amplified, it contained a 1305 bp Open Reading Frame (ORF) which encoded 434 amino acids. The molecular mass of the deduced amino acid sequences was 56 kD. BLAST analysis showed that it shared high identities (91.0-99.0%) with sip sequences of other GBS registered in GenBank while lower identities (<50%) was found compared with other species of *Streptococcus*. The deduced amino acid homology was 90.2-98.8%.

CONCLUSION

In this study, *Sip* gene was amplified by PCR type of bovine *S. agalactiae* and the Bioinformatics Software was used to analyze variety of the genetics, in order to deeply study and provide basic information of bovine *S. agalactiae* and provide scientific basis for study of molecular characteristics and different sources of non-separation of *Streptococcus Sip* gene and provide new ideas for the development of dominant antigen screening cross protection strong and novel vaccines.

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