Veterinary Microbiology 225 (2018) 120–124

Contents lists available at ScienceDirect

Veterinary Microbiology

journal homepage: www.elsevier.com/locate/vetmic

Short communication

Prevalence and diversity of M-like protein (SCM) gene in *Streptococcus canis* isolates from diseased companion animals in Japan: Implication of SCM allele

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ARTICLE INFO

Keywords:
Companion animals
Streptococcus canis
M-like protein (SCM) gene
SCM allele
Japan

ABSTRACT

Streptococcus canis (*Sc*)-origin M-like protein (SCM) binds to plasminogen and immunoglobulin G and facilitates anti-phagocytic properties. We aimed to determine the prevalence and diversity of the *scm* gene in *Sc* isolates from diseased companion animals in Japan and to propose potential SCM alleles of amino acid (AA) sequences. We collected β-hemolytic streptococci from diseased animals with host information nationwide in 2015 and 2017. After *Sc* identification and *scm* gene amplification and sequencing, the gene's prevalence and relationship between its presence and host information were determined. Furthermore, phylogenetic trees of AA sequences were constructed, and classification and distribution of SCM alleles based on variations of AA sequences were conducted. The *scm* detection rates were 70.6% (n = 48, 2015) and 82.9% (n = 97, 2017). There was a relationship between *scm* presence and Tokyo in 2015 and 2017. We found an association between *scm* detection and dogs in 2017 alone. Major sequence sizes were 1311 bp, 1308 bp, and 1305 bp. Using the phylogenetic trees of AA sequences, we confirmed shared positions of five identical sequence patterns in both periods. Nine SCM alleles were determined with six signal-peptide types. Most prevalent alleles were type 1, type 2, and type 4 in both periods. Our observations suggest prevalence and diversity of *scm* in animal-origin *Sc* isolates in Japan.

1. Introduction

Streptococcus canis (*Sc*), which was first reported in 1986 (Devriese et al., 1986), is an emerging zoonotic bacterium with increasing importance and can sometimes cause self-limiting dermatitis, as well as severe diseases including arthritis, streptococcal toxic shock syndrome, necrotizing fasciitis, septicemia, and pneumonia in companion animals with the underlying conditions (Iglauer et al., 1991; DeWinter and Prescott, 1999; Lamm et al., 2010). This microorganism can also infect humans who may have been in close contact with animals and cause either local (ulcer infection) or systemic diseases (bacteremia, septicemia, and endocarditis) (Galpérine et al., 2007; Amsallem et al., 2014).

Sc strains appear as large gray-white-colored smooth colonies surrounded by zones of complete hemolysis when cultured on sheep blood agar plates. Furthermore, Lancefield grouping classifies *Sc* as group G Streptococcus based on the composition of carbohydrate antigens in the cell wall. In healthy dogs, *Sc* is observed as a resident flora of the oropharynx, skin, genital urinary tract, and anus (Devriese et al., 1992). The *Sc*-derived M-like protein (SCM) binds to plasminogen and immunoglobulin G and facilitates anti-phagocytic properties (Fulde et al., 2011, 2013; Bergmann et al., 2017).

Timoney et al. (2017) documented four SCM alleles of *Sc* isolates from diseased and healthy cats and described that the type 1 allele was predominant in diseased cats. The purpose of this study was to determine the prevalence and diversity of *scm* in the isolates from the diseased companion animals nationwide. We also represent the proposal concerning potential alleles of deduced SCM amino acid (AA) sequences.

2. Materials and methods

2.1. Collection of bacterial isolates and animal information

To examine the causative bacterial agents in the clinical specimens by veterinary practitioners, the specimens were immediately sent to the

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https://doi.org/10.1016/j.vetmic.2018.09.021
Received 18 April 2018; Received in revised form 18 September 2018; Accepted 18 September 2018

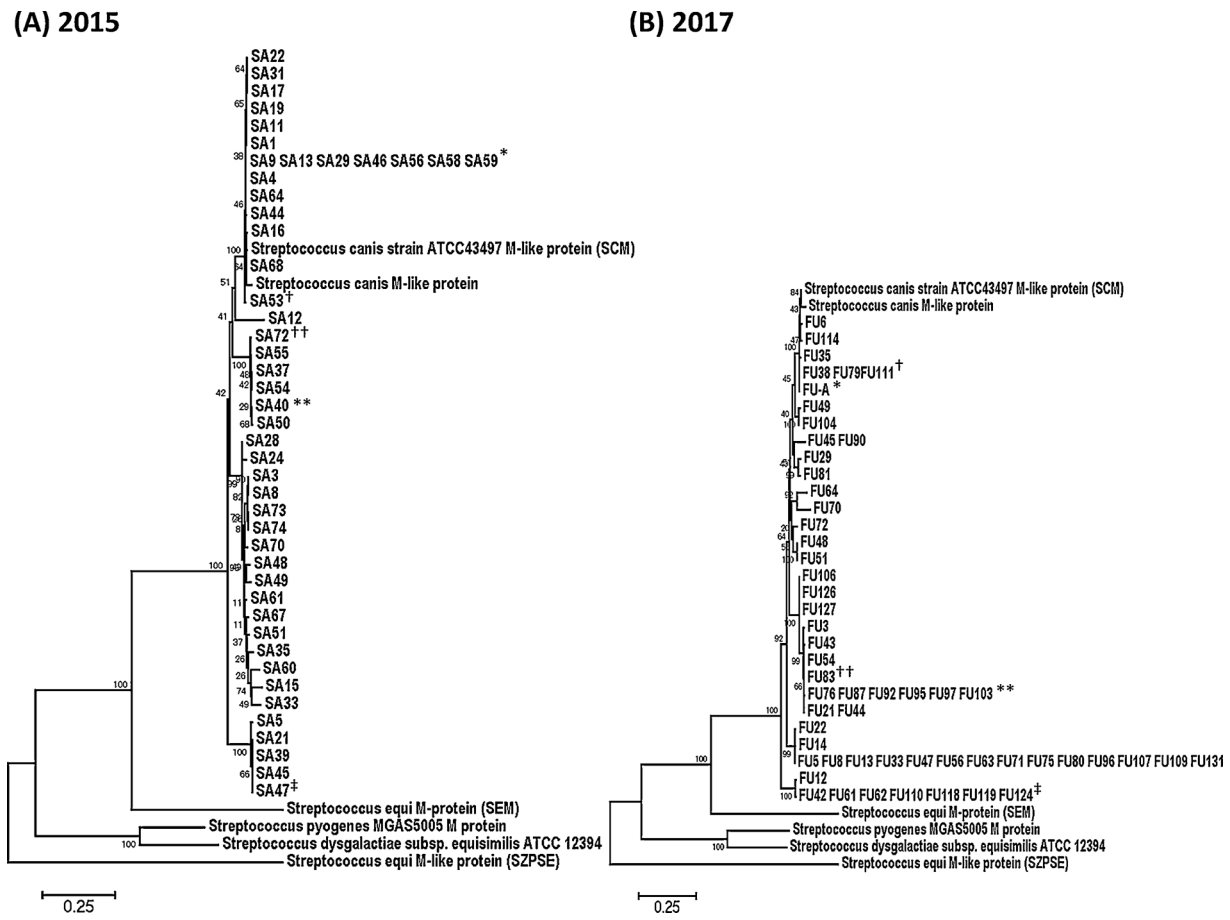

Fig. 1. Phylogenetic trees of *S. canis* M-like protein in 2015 (A) and 2017 (B) by Neighbor-Joining method. The symbols indicate identical sequences occurring in 2015 and 2017. FU-A includes FU4, FU10, FU11, FU15, FU18, FU19, FU20, FU23, FU24, FU26, FU28, FU31, FU36, FU39, FU40, FU41, FU46, FU50, FU52, FU55, FU57, FU59, FU67, FU74, FU77, FU82, FU84, FU85, FU86, FU89, FU94, FU101, FU102, FU105, FU108, FU112, FU115, FU120, FU123, FU125, and FU128.

Sanritsu Zelkova Veterinary Laboratory, with request sheets including the animal information (animal species, gender, age, clinical specimen, date collected, and Japanese prefecture where the practitioners worked). These specimens were taken from diseased companion animals, with significant symptoms that were found by their pet owners or veterinary clinicians, which visited either clinics or hospitals during two study periods (for approximately 2 months from beginning of April to end of May, in 2015 and 2017). The specimens were derived from either sterile (i.e. blood, joint fluid) or non-sterile samples (i.e. ear discharge, open pus). Each specimen was inoculated on a sheep blood agar plate, which was incubated in 5% CO_2 at 35 °C for 24 h. Gray-white colonies with β-hemolytic activity were subjected to latex agglutination testing with antisera specific for the classification of Lancefield carbohydrate antigens (Seroiden Strepto Kit, Eiken Chemical Co., Ltd., Tokyo, Japan). All the isolates (one isolate per animal) were stored at −70 °C to −80 °C until they were processed. The stored streptococci with the animal information were sent to our laboratory for further genotypic and phenotypic analyses.

2.2. Determination of species identification

We identified the β-hemolytic isolates at the *Sc* species level based on 16S rRNA sequencing data with the detection of *Sc*-specific gene, *cfg* encoding the CAMP-factor (Tsuyuki et al., 2017). To confirm the biochemical properties, rapid ID 32 STREP API V4.0 (SYSMEX bioMérieux Co., Ltd., Tokyo, Japan) was performed on each isolate. The criteria to accept the identification data using either 16S rRNA sequencing or the rapid ID 32 STREP API were that the isolate was identified as the only one choice by either ≥98.7% similarity to the 16S rRNA sequence of the type strain (ATCC 43496) or ≥80% probability.

2.3. Amplifying and sequencing scm nucleotide sequences

Bacterial DNA was extracted by the boiling method (97 °C for 10 min) using bacterial suspension in TE buffer. First, we performed polymerase chain reaction (PCR)-based amplification of *scm* using the internal primer set all-canis_fwd/all-canis_rev, yielding an amplicon of approximately 1000 bp (Fulde et al., 2011). After confirming the PCR product, we did the full-length amplification using the external primer set either M-SCAF2/M-SCAR3 or M-SCAF2/M-SCAR4, resulting in an amplicon of either 1499 or 1658 bp (Timoney et al., 2017). The same primers were used for both amplification and direct sequencing after the PCR product purification to determine the full-length nucleotide sequences. We investigated the variations of *scm* sequences, its prevalence, and relationship between the *scm* possession and animal information during both periods.

2.4. Construction of phylogenetic trees of deduced SCM AA sequences

We constructed phylogenetic unrooted trees of the deduced AA sequences based on the determined nucleotide sequences, using the Neighbor-Joining method (Saitou and Nei, 1987). Briefly, after the associated taxa clustered together in the bootstrap test (1000 replicates) (Felsenstein, 1985), the tree was drawn to scale, with branch lengths in the same units as those of the evolutionary distances. These distances were computed using the Poisson correction method (Zuckerkandl and Pauling, 1965). These evolutionary analyses were conducted in MEGA7