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ORIGINAL ARTICLE

Genotyping and study of the *pauA* and *sua* genes of *Streptococcus uberis* isolates from bovine mastitis

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KEYWORDS
Bovine mastitis;
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Abstract This study aimed to determine the clonal relationship among 137 *Streptococcus uberis* isolates from bovine milk with subclinical or clinical mastitis in Argentina and to assess the prevalence and conservation of *pauA* and *sua* genes. This information is critical for the rational design of a vaccine for the prevention of bovine mastitis caused by *S. uberis*. The isolates were typed by random amplified polymorphic DNA (RAPD) analysis and by pulsed-field gel electrophoresis (PFGE). The 137 isolates exhibited 61 different PFGE types and 25 distinct RAPD profiles. Simpson's diversity index was calculated both for PFGE (0.983) and for RAPD (0.941), showing a high discriminatory power in both techniques. The analysis of the relationship between pairs of isolates showed 92.6% concordance between both techniques indicating that any given pair of isolates distinguished by one method tended to be distinguished by the other. The prevalence of the *sua* and *pauA* genes was 97.8% (134/137) and 94.9% (130/137), respectively. Nucleotide and amino acid sequences of the *sua* and *pauA* genes from 20 *S. uberis* selected isolates, based on their PFGE and RAPD types and geographical origin, showed an identity between 95% and 100% with respect to all reference sequences registered in GenBank. These results demonstrate that, in spite of *S. uberis* clonal diversity, the *sua* and *pauA* genes are prevalent and highly conserved, showing their importance to be included in future vaccine studies to prevent *S. uberis* bovine mastitis.
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PALABRAS CLAVE
Mastitis bovina;
Tipificación molecular;
Streptococcus uberis;
PauA;
SUAM

Genotipificación y estudio de los genes *pauA* y *sua* de aislamientos de *Streptococcus uberis* de mastitis bovina

Resumen Este estudio pretendió determinar la relación clonal entre 137 aislamientos de *S. uberis* obtenidos de leche de bovinos con mastitis clínica o subclínica en la Argentina, como así también la prevalencia y la conservación de los genes *sua* y *PauA* entre dichos aislamientos. Esta información es crítica para el diseño racional de una vacuna que prevenga la mastitis bovina por *S. uberis*. Los aislamientos se tipificaron molecularmente por amplificación al azar del ADN polimórfico (RAPD) y mediante electroforesis de campos pulsados (PFGE). Los 137 aislamientos mostraron 61 pulsotipos mediante PFGE y 25 tipos de RAPD diferentes. Los índices de Simpson calculados fueron 0,983 por PFGE y 0,941 por RAPD; esto evidencia el elevado poder discriminatorio de ambas técnicas. El análisis de la relación entre pares de aislamientos mostró un 92,6 % de concordancia entre ambas técnicas, lo que indica que cualquier par de aislamientos que fue distinguido por un método tendió a ser distinguido por el otro. La prevalencia de los genes *sua* y *puaA* fue del 97,8 % (134/137) y 94,9 % (130/137), respectivamente. Las secuencias de nucleótidos y de aminoácidos codificados por los genes *sua* y *pauA* de los 20 aislamientos de *S. uberis* seleccionados sobre la base de su tipo de PFGE y RAPD y origen geográfico tuvieron un porcentaje de identidad de entre 95 % y 100 % con respecto a todas las secuencias de referencia registradas en GenBank. Estos resultados demuestran que, a pesar de la diversidad clonal de *S. uberis*, los genes *sua* y *pauA* son prevalentes y están altamente conservados y deberían ser incluidos en futuros estudios de vacunas para prevenir mastitis bovina causada por *S. uberis*.
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Introduction

Bovine mastitis is one of the most costly diseases of dairy cattle as a consequence of antibiotic treatment expenses, decreased milk production and quality, and increased animal replacement rate[6,30]. In Argentina, milk yield losses in cows suffering from mastitis were reported to be of about $4.3/cow/days[41]. Bovine intramammary infections (IMI) are caused by both contagious and environmental bacteria. *Streptococcus uberis* is one of the most prevalent environmental pathogens associated with subclinical and clinical IMI both in lactating and non-lactating cows[7,27]. In addition, this pathogen can persist in the mammary gland causing chronic IMI[47]. Traditional control procedures based on milking time hygiene and antibiotic therapy are considered adequate to reduce incidence of most contagious pathogens, but are often insufficient for the control of IMI caused by *S. uberis*[20]. Consequently, the interest has focused on the development of immunoprophylactic strategies. However, a significant obstacle in the design of an effective vaccine is the high level of genetic variability of different isolates of *S. uberis*, frequently involving virulence factor genes[18,31]. In this context, epidemiological studies of regional isolates are extremely useful to detect the frequency and distribution of bacterial types associated with IMI and to identify target molecules for the development of immunogens and therapeutic agents. Methods based on DNA analysis including restriction fragment length polymorphism (RFLP), random amplified polymorphic DNA (RAPD), pulsed field gel electrophoresis (PFGE), and multilocus sequence typing (MLST) have been successfully applied to fulfill this need. PFGE is the most discriminatory method for typing bacteria[22], whereas RAPD is a more straightforward method given its relatively lower costs, execution time demanded, less expensive equipment requirements and sensitivity. Both methods have been widely used to study the genetic variability of many bacterial species, including important human pathogens[22,37].

Several *S. uberis* virulence factors have been described[16,20,23,24]. Among them, plasminogen activator A (PauA)[35] and *S. uberis* adhesion molecule (SUAM)[2] have shown potential as protective immunogens. The former is a protease capable of activating plasmin, which in turn, degrades proteins producing small peptides and free amino acids used by bacteria as a nitrogen source. This factor has been related to early mammary gland colonization[21] while SUAM both to adherence and internalization, through its binding to lactoferrin[28], and to bacterial persistence in bovine mammary epithelial cells *in vitro*[2].

The wide genetic diversity observed in *S. uberis* indicates that virulence is not associated with any specific molecular type[43]. Therefore, immunologic prevention strategies against this organism should be directed to a multitude of different factors included in field isolates from individual herds. Nevertheless, the difficulty associated with *S. uberis* genetic variability can be currently overcome through bioinformatic tools that allow to analyze DNA sequence encoding genes from virulence factors which are found in a large number of isolates. Thus, epidemiological studies on gene distribution together with their sequence analysis will contribute to identify potential antigens as vaccine components.

The main objectives of this work were to determine the clonal relationship between *S. uberis* isolates from milk

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