Microbial Pathogenesis 104 (2017) 278–286

Contents lists available at ScienceDirect

Microbial Pathogenesis

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

Evaluation of the biofilm forming ability and its associated genes in *Staphylococcus* species isolates from bovine mastitis in Argentinean dairy farms

Verónica Felipe [a, b], Carolina A. Morgante [a], Paola S. Somale [a], Florencia Varroni [a], María L. Zingaretti [a], Romina A. Bachetti [a], Silvia G. Correa [c], Carina Porporatto [a, b, *]

[a] Instituto Académico Pedagógico de Ciencias Básicas y Aplicadas (IAPCByA), Universidad Nacional de Villa María (UNVM), Villa María, Argentina
[b] Centro de Investigaciones y Transferencia de Villa María (CIT-VM), Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Universidad Nacional de Villa María (UNVM), Villa María, Argentina
[c] Centro de Investigaciones en Bioquímica Clínica e Inmunología (CIBICI)-Consejo Nacional de Investigaciones Científicas y Tecnológicas (CONICET), Departamento de Bioquímica Clínica, Facultad de Ciencias Químicas, Universidad Nacional de Córdoba, Córdoba, Argentina

ARTICLE INFO

Article history:
Received 18 March 2016
Received in revised form
18 January 2017
Accepted 24 January 2017
Available online 25 January 2017

Keywords:
Staphylococcus
Biofilm
Dairy cow
Mastitis

ABSTRACT

Staphylococcus aureus and coagulase-negative staphylococci (CNS) are important causes of intramammary infection in dairy cattle, and their ability to produce biofilm is considered an important virulence property in the pathogenesis of mastitis. However, the published date on mechanisms and factors involved in infection persistence in the mammary gland remains unclear. The aim of this study was to investigate whether the main *Staphylococcus* species involved in bovine intramammary infections possess specific characteristics that promote colonization of the udder. We evaluated the biofilm-forming ability and distribution of adhesion- and biofilm-associated genes of *Staphylococcus* spp. isolated from bovine mastitis infected animals in Argentinean dairy farms. For this purpose, the phenotypic biofilm formation ability of 209 *Staphylococcus* spp. from bovine mastitis was investigated. All isolates produced biofilm *in vitro*, being 35,0% and 45,0% of the 127 *S. aureus* or 51,0% and 29,0% of the 82 CNS strong and moderate biofilm producers respectively. All *S. aureus* samples were PCR-positive for *ica*A, *ica*D, *clf*A, *clf*B and *fnbp*A genes, 76.3% were positive for *fnbp*B gene and 11.0% were positive for *bap* gene. In CNS isolates, the positive rates for *ica*A and *ica*D were 73.2%, while for *clf*A, *clf*B, *fnbp*A *fnbp*B and *bap* genes the percentage were lower. The results demonstrate that in *Staphylococcus* spp. biofilm formation, the polysaccharide and the adhesion- and biofilm-associated genes are of overall importance on bovine mastitis in Argentina. Therefore, future works should focus on these pathogenic specific factors for the development of more effective therapies of control, being essential to consider the ability of isolates to produce biofilm.

© 2017 Elsevier Ltd. All rights reserved.

1. Introduction

Bovine mastitis is an inflammation of the mammary glands in dairy cattle, usually caused by bacteria. It leads to significant economic losses due to reduced milk production, increased use of drugs and animal morbidity and mortality [1]. In the last years, *Staphylococcus aureus* has been described as a major pathogen responsible for bovine mastitis [2], whereas coagulase-negative staphylococci (CNS) have been increasingly isolated from clinical and subclinical mastitis [3–5]. In dairy cows of Córdoba (Argentina), the most frequently isolated pathogens are CNS (52.1%) followed by *S. aureus* (21.3%) [6].

The ability of *Staphylococcus* spp. to form biofilm is one of the virulence factors that facilitate the adhesion and colonization on the mammary gland epithelium, a fact that leads to recurrent or persistent infections [7,8]. Biofilm is defined as a structured community of bacterial cells enclosed in a self-produced matrix attached to biotic or abiotic surfaces and the ability to form biofilm is considered a universal trait of microorganisms [9]. Biofilm offers

* Corresponding author. Centro de Investigaciones y Transferencia de Villa María (CIT-VM), Universidad Nacional de Villa María (UNVM), Arturo Jauretche 1555, Campus Universitario, Villa María (5900), Argentina.
E-mail address: cporporatto@unvm.edu.ar (C. Porporatto).

http://dx.doi.org/10.1016/j.micpath.2017.01.047
0882-4010/© 2017 Elsevier Ltd. All rights reserved.

protection against hostile environments, such as the immune response and bactericidal concentrations of antibiotics or disinfectants [10]. In the process of staphylococcal biofilm formation, the accumulation and development of a mature stage depend mainly on the polysaccharide intercellular adhesions (PIA) that promote bacterial accumulation, especially polysaccharide poly-*N*-succinyl-β-1-6 glucosamine (PNAG). PNAG biosynthesis is regulated by enzymes encoded by the *ica*ADBC operon [11]. PIA/PNAG are probably the most important components of the extracellular matrix in staphylococci, although there is evidence of *in vitro* and *in vivo* staphylococcal biofilm formation without PIA/PNAG [12]. Bacterial surface proteins contribute significantly to adhesion, and several key proteins have been identified as important in staphylococcal biofilm formation [13]. These include proteins involved in bacterial adhesion, such as clumping factor (ClfA and ClfB) [14] and fibronectin-binding proteins (FnbpA and FnbpB) [15], and particularly the biofilm-associated protein (Bap) [16]. The Bap protein was described as a specific pathogenic factor of cattle because it has only been identified in isolates from bovine intramammary infections [17,18].

Several studies evaluated biofilm formation and the presence of various putative virulence genes, suspected of playing a role in the pathogenicity of staphylococci as factors associated with intramammary infections [4]. Recently it has been reported the production of biofilm by staphylococci strains causing mastitis, isolated from different regions of the world [7,19–22]. In Argentina, although there are some genotypic studies on CNS [23], the ability of *S. aureus* causing mastitis to produce biofilm has not been investigated thoroughly. Pathogen-specific risk factors and associated control measures need to be identified due to the pathogen-related variation in epidemiology and their effect on future performance [24]. However, the question of whether these factors play an important role in biofilm formation remains unanswered. Therefore, the aim of the present study was to test the biofilm-forming ability of 209 *Staphylococcus* strains isolated from animals with bovine mastitis in Argentinean dairy farms and to analyze the distribution of biofilm-associated genes. Studies on the ability of *Staphylococcus* to form biofilm and on the underlying mechanisms may provide new ideas for the prevention and treatment of bovine mastitis.

2. Materials and methods

2.1. Collection of bacterial isolates

A total of 209 *Staphylococcus* strains were isolated from composite samples containing milk from all quarters of each cows with mastitis. Isolates were obtained from 32 commercial dairy farms located in Buenos Aires and Córdoba provinces (a major dairy producing area from Argentina) and samples of no more than 10% of the dairy cows were collected. Staphylococci were identified presumptively based on colony morphology, Gram's stain, catalase test and hemolysis on blood agar. *S. aureus* isolates were differentiated from CNS isolates based on coagulase production on rabbit plasma and *S. aureus* isolates were screened for growth on Chromagar™ Staph aureus (CHROMagar, Paris, France), respectively. As positive or negative controls, *S. aureus* ATCC 25923 (biofilm-forming) and *Staphylococcus epidermidis* ATCC12228 (biofilm-negative) were used [25]. The *S. aureus* strain V329, a bovine subclinical mastitis isolate with strong biofilm production phenotype, kindly provided by Dr. Lasa (Instituto de Agrobiotecnología, Pamplona, Spain), was used as a positive control. Bacteria were stored at −70 °C and freshly cultured in tryptic soy broth (TSB) (Britania, Buenos Aires, Argentina) before experiments.

2.2. Isolation of bacterial DNA

Genomic DNA was extracted using the Wizard DNA Purification kit (Promega, Madison, WI, USA) according to the manufacturer's instructions, and 4 mg/ml lysostaphin solution (Sigma, St Louis, MO, USA) was added to the cell suspension, for enzymatic lysis of cell wall by hydrolysis of peptidoglycan, at 37 °C for 1 h. DNA was suspended in 100 μl of RNase-free water (Biodynamics, Buenos Aires, Argentina) and frozen at −20 °C.

2.3. S. aureus confirmation by PCR

All coagulase positive isolates were confirmed as *S. aureus* by using the PCR analysis to amplify the 16S-23S rRNA intergenic spacer region as previously described by Forsman et al. [26]. The primers used in the PCR assays, as well as the expected amplified product size, are listed in Table 1.

2.4. Identification of CNS species by MALDI-TOF MS

CNS species identification was performed by matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) by Instituto de Biotecnología y Biología Molecular (CONICET, La Plata, Argentina). MALDI-TOF MS is proposed as a powerful tool for precise identification and discrimination of *Staphylococcus* species based on the proteomic specific profiles [27]. Briefly, of the 82 randomly selected strains, one or two colonies of a fresh overnight culture grown on TSA were suspended in ethanol (70%). A solution of 70% formic acid and acetonitrile 1:1 was added to the pellet. Samples were processed in an Ultraflex MALDI-TOF mass spectrometer (Bruker Daltonics, Bremen, Germany) with flex control software (Bruker Daltonics) operated in linear mode and equipped with a 337-nm nitrogen laser. To prepare the MALDI target plate, 1 μl of each bacterial extract was spotted onto a 384-spot target plate (polished stainless steel, Bruker Daltonics) and dried at room temperature. The dried sample spot was overlaid with 1 μl of a matrix solution, consisting of α-cyano-4-hydroxycinnamic acid diluted in a solution of 50% acetonitrile and 2.5% trifluoroacetic acid. The Bruker bacterial test standard (BTS) (Bruker Daltonics) was used for mass calibration and for instrument parameter optimization. Mass spectral data were collected within the m/z range of 2.000–20.000 and the data were acquired using the FlexControl software 3.3 (Bruker Daltonics). Biotyper 3.0 software (Bruker Daltonics) was used to process the raw spectra acquired by the Ultraflex. Data were analyzed using the built-in main spectra projection feature of the Biotyper software, which is a proprietary algorithm for spectral pattern matching that produces a logarithmic score from 0 to 3. The peak lists were compared with each entry in the Biotyper database, which contained 3.995 references, using the standard parameters of the pattern-matching algorithm. An identification score $\geq$2.00 was considered high-confidence identification to the species level, whereas scores of 1.70–1.99 were considered intermediate confidence genus-level identification only. Scores of <1.70 were considered unacceptable identification, according to the manufacturer's recommendations. Isolates with an identification score <1.70 were considered other CNS species [27].

2.5. Biofilm production

To determine the potential of an isolate to form biofilm we used the microtiter plate-based assay and the biofilm formation on abiotic surfaces was quantified as described elsewhere [28], with some modifications. Briefly, overnight cultures of each isolate were diluted (1:40) in fresh TSB. Aliquots (200 μl) of each prepared

Download English Version:

https://daneshyari.com/en/article/5674018

Download Persian Version:

https://daneshyari.com/article/5674018

Daneshyari.com