Mar 25, 2019 Version 2

Polymerase Chain Reaction - Comparative genomics of Staphylococcus aureus associated with subclinical and clinical bovine mastitis (Rocha et al., 2019) V.2

DOI
dx.doi.org/10.17504/protocols.io.zhpf35n

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Protocol Citation: Lis LSR Rocha 2019. Polymerase Chain Reaction - Comparative genomics of Staphylococcus aureus associated with subclinical and clinical bovine mastitis (Rocha et al., 2019). **protocols.io**
https://dx.doi.org/10.17504/protocols.io.zhpf35n

Protocol status: Working
We use this protocol and it's working

Created: March 25, 2019

Last Modified: March 25, 2019

Protocol Integer ID: 21775

Keywords: comparative genomics of staphylococcus aureus, pathogenesis of bovine mastitis, bovine mastitis, clinical bovine mastitis, staphylococcus aureus, causing clinical mastitis, clinical mastitis, mastitis outcome, genes present in the subclinical strain, causing subclinical infection, subclinical infection, clinical isolates from the subclinical one, virulence factor, comparative genomic, clinical isolate, subclinical strain, absence of virulence factor, bacteria, subclinical mastit, strain, virulence, comparative genomic analysis, several nonsynonymous single nucleotide polymorphism, associated gene, characterized strain, pathogenesi, nucleotide sequence, polymerase chain reaction

Abstract

Comparative genomics of *Staphylococcus aureus* associated with subclinical and clinical bovine mastitis (Rocha et al., 2019)

Many efforts have been made to understand the pathogenesis of bovine mastitis to reduce losses and promote animal welfare. *Staphylococcus aureus* may cause bovine clinical mastitis, but it is mainly associated with subclinical infection, which is usually persistent and can easily reoccur. Here, we conducted a comparative genomic analysis between four strains of *S. aureus* causing subclinical infection (Sau170, 302, 1269, 1364), previously sequenced by our group, and two well-characterized strains causing clinical mastitis (N305 and RF122) to find differences that could be linked to mastitis outcome. A total of 146 virulence-associated genes were compared and no appreciable differences were found between the bacteria. However, several nonsynonymous single nucleotide polymorphisms (SNPs) were identified in genes present in the subclinical strains when compared to RF122, especially in genes encoding host immune evasion and surface proteins. The comparison of orthologous genes using OrthoMCL identified a membrane transporter in the genomes of the bacteria belonging to the subclinical group, but this finding was not confirmed by polymerase chain reaction (PCR) on a collection of field isolates of *S. aureus* associated with clinical or subclinical mastits. The secreted and surface proteins predicted by different in silico tools were compared through multidimensional scaling analysis, revealing a high degree of similarity among the six strains. However, differences were seen in the nucleotide sequences of a gene that codes for a hypothetical protein (cI3309) and a lipoprotein (cI3700). These findings were also analyzed by PCR on DNA extracted from field isolates of *S. aureus*. The lipoprotein, but not the hypothetical protein, was able to separate the clinical isolates from the subclinical ones. These results show that sequence variation among bovine *S. aureus*, and not only the presence/absence of virulence factors, is an important aspect to consider when comparing isolates causing different mastitis outcomes

Materials

50 ng of total DNA, 1U of Taq DNA polymerase Cellco Biotec, 0.2 µM of each primer, 0.2 mM deoxynucleotide triphosphate mixture, 1X reaction buffer containing 2.0 mM MgCl2, extra 1.0 mM MgCl2, and Milli-Q water to increase the reaction volume to a final volume of 25 µL.

The extra 1 mM $MgCl_2$ was excluded from thePCR reactionsthat contained the primers LipoP-F-CS/LipoP-R-C.

Table 1 - Primer Sequences for primers used in this Protocol

	cl3309subF	TGTTGTAGGAGGAACAATCC
	cl3309subR	TTCTAATGTCAGCAACATGC
	cl3309cliF	GCTATTCCTAGATGCACT
	cl3309cliR	TTTTAAGTATGACATGAATG
	cl3316F	ACGCAAAACCCTTTACTAGT
	cl3316R	GCAACAACTAGTAGGAGTGA
	LipoP-F-CS	GYTTTGCGAAAACGTTAGAYATGTA
	LipoP-R-C	TGCCTTCATCATTAATTGGACCAATC
	LipoP-F-CS	GYTTTGCGAAAACGTTAGAYATGTA
	LipoP-R-CS	GGTAAAYTCAATGTYCTTATRTCC

Troubleshooting

primers cI3309sub F/R

1 Initial denaturation: 95.0 °C for 5 min;

2 35 cycles of denaturation at 95.0 °C for 45 s,

3 Anealing: 55 °C for 45s

4 Extension: 72 °C for 45 s

5 final extension at 72.0 °C for 10 min

primers cI3316F/R

6 initial denaturation: 95.0 °C for 5 min;

7 35 cycles of denaturation at 95.0 °C for 45 s,

8 Annealing: 55 °C for 45 s

9 Extension: 72 °C for 45 s

10 final extension at 72.0 °C for 10 min.

primers cI3700 - LipoP FCS/RC

11 initial denaturation: 95.0 °C for 5 min;

12 35 cycles of denaturation at 95.0 °C for 45 s,

13 Annealing: 54 °C for 45 s

14 Extension: 72 °C for 45 s

15 final extension at 72.0 °C for 10 min.

cl33009cli F/R

16 initial denaturation: 95.0 °C for 5 min;

17 35 cycles of denaturation at 95.0 °C for 45 s,

18 Annealing: 45 °C for 45 s

19 Extension: 72 °C for 30 s

20 final extension at 72.0 °C for 10 min.

primers cl3700 - LipoP FCS/RCS

21 initial denaturation: 95.0 °C for 5 min;

22 35 cycles of denaturation at 95.0 °C for 45 s,

23 Annealing: 50 °C for 45 s

24 Extension: 72 °C for 1min

25 final extension at 72.0 °C for 10 min.

Analyzing the amplified fragments

26 Analyze the amplicons by electrophoresis in 1X Tris-acetate-EDTA on a 1.0% agarose gel and visualize
imagen under UV light after staining with 2 mg.ml-1 ethidium bromide.