Short communication: Search for superantigen genes in coagulase-negative staphylococci isolated from bovine milk in Canada

A. Corbeil, J. Labrie, C. Goetz, S. Dufour, I. Doghri, L. Rivière, and M. Jacques*

Regroupement de recherche pour un lait de qualité optimale (Op+Lait), Faculté de médecine vétérinaire, Université de Montréal, St-Hyacinthe, Québec, Canada J2S 2M2

ABSTRACT

The objective of the present study was to look for genes encoding for superantigens in bovine coagulase-negative staphylococci (CNS) isolated from milk in Canada. We screened by PCR 71 bovine CNS isolates, obtained from the Mastitis Pathogen Culture Collection managed by the Canadian Bovine Mastitis and Milk Quality Research Network (St-Hyacinthe, QC, Canada), for the presence of 13 superantigen genes. Our results indicate that these CNS isolates did not have any of the 13 superantigen genes screened for in the present study. Thus, prevalence of those genes in CNS from milk is expected to be quite low in Canada.

Key words: coagulase-negative staphylococci, enterotoxin, superantigen, polymerase chain reaction

Short Communication

Coagulase-negative staphylococci are the bacteria most frequently isolated from bovine milk worldwide (Condas et al., 2017). They are a heterogeneous group of numerous species with vast differences in pathogenicity, ecology, and epidemiology (Cameron et al., 2017). Among the 20 CNS species isolated from bovine milk samples in Canada, the 5 most common species found were *Staphylococcus chromogenes*, *Staphylococcus simulans*, *Staphylococcus xylosus*, *Staphylococcus haemolyticus*, and *Staphylococcus epidermidis* (Fry et al., 2014; Condas et al., 2017).

Our group has studied biofilm formation by bovine CNS (Tremblay et al., 2013; Goetz et al., 2017), but, overall, not much is known about their virulence factors. It is well known that *Staphylococcus aureus* (coagulase-positive staphylococci) express various virulence factors, including secreted toxins. These toxins can be divided into 3 major groups: the pore-forming toxins (e.g., hemolysins, leukotoxins), the exfoliative toxins, and the

superantigens (SAg; Oliveira et al., 2018). There are 26 staphylococcal SAg, including enterotoxins (e.g., staphylococcal enterotoxin A, SEA), enterotoxin-like proteins (proteins that have no demonstrated emetic potential in nonhuman primate models; e.g., staphylococcal enterotoxin-like K, SELK), and the toxic shock syndrome toxin (Fisher et al., 2018). These SAg interact with both antigen-presenting cells and T-lymphocytes, directly cross-linking the major histocompatibility class II antigen and the T-cell receptor (Cavaillon, 2018). This results in a massive proinflammatory cytokine response (Cavaillon, 2018). In addition, staphylococcal enterotoxins are highly resistant to heat and will resist treatments that destroy bacterial cells (Le Loir et al., 2003; Podkowik et al., 2013). For example, staphylococcal enterotoxins may persist in milk after pasteurization (Necidova et al., 2016). These potent exotoxins are also secreted by staphylococcal species other than *S. aureus* (Podkowik et al., 2013; Tuffs et al., 2018). Indeed, genes encoding for enterotoxins and SAg have been reported in bovine CNS isolates from different countries (Park et al., 2011; Fijalkowski et al., 2014; Rall et al., 2014; Bertelloni et al., 2015). Because the presence of SAg might affect milk quality and represent a public health threat, the objective of the present study was to look for genes encoding for SAg in bovine CNS isolated from milk in Canada.

Coagulase-negative staphylococci isolates were obtained from the Mastitis Pathogen Culture Collection (Reyher et al., 2011) managed by the Canadian Bovine Mastitis and Milk Quality Research Network (St-Hyacinthe, QC, Canada; Table 1). These CNS isolates originated from milk from clinical and subclinical mastitis cases as well as from milk from apparently normal mammary glands. They were isolated from 65 cows in 41 herds across 7 provinces. They were assigned to a species by amplifying and sequencing the staphylococcal *rpoB* gene (Fry et al., 2014) and using MALDI-TOF MS analysis (Cameron et al., 2017). *Staphylococcus aureus* control strains were also included in the study (Table 2). Genomic DNA was isolated as previously described by Tremblay et al. (2013). The

Received September 5, 2018.

Accepted November 21, 2018.

*Corresponding author: mario.jacques@umontreal.ca

Table 1. Coagulase-negative staphylococci isolates used in the present study

CNS species	Source	No. of isolates
<i>Staphylococcus chromogenes</i>	S. Dufour ¹	25
<i>Staphylococcus epidermidis</i>	S. Dufour	5
<i>Staphylococcus haemolyticus</i>	S. Dufour	5
<i>Staphylococcus simulans</i>	S. Dufour	10
<i>Staphylococcus xylosus</i>	S. Dufour	26

¹Mastitis Pathogen Culture Collection, Université de Montréal, St-Hyacinthe, QC, Canada.

presence of 13 SAg genes was detected by PCR with primers described by Park et al. (2011; Table 3). The PCR products were sequenced (Plateforme d'analyses génomiques, IBIS, Université Laval, QC, Canada) to verify the identity of the amplicons.

We first screened by PCR 71 bovine CNS isolates for the presence of 13 SAg genes with appropriate negative and positive controls. This initial screen revealed the presence of 27 amplicons [*selo* (n = 9), *sec* (n = 8), *seg* (n = 5), *seb* (n = 4), and *sell* (n = 1)]. No amplicons were obtained for the *sea*, *sed*, *selk*, *selm*, *seln*, *selp*, *selq*, and *tst* genes. Sequencing results indicated that the positive controls gave the expected DNA sequences, but all 27 amplicons from the CNS isolates were the result of nonspecific amplifications.

Our results indicate that the 71 CNS isolates did not have any of the 13 SAg genes screened for in the present study. With that sample size, a 95% confidence interval of 0.0 to 5.1% can be computed, thus indicating with 95% certainty that the prevalence of those genes in CNS from milk is lower than 5.1% in Canada. Our

Table 2. *Staphylococcus aureus* control strains used in the present study

Control strain	Source	Genes
<i>S. aureus</i> N315	F. Malouin ¹	<i>sec</i> , <i>seg</i> , <i>sell</i> , <i>selm</i> , <i>seln</i> , <i>selo</i> , <i>selp</i> , <i>tst</i>
<i>S. aureus</i> Col	F. Malouin	<i>seb</i> , <i>selk</i> , <i>selq</i>
<i>S. aureus</i> CMRSA-2	S. Moineau ²	<i>sed</i>
<i>S. aureus</i> CMRSA-4	S. Moineau	<i>sea</i>
<i>S. aureus</i> RN4220	F. Malouin	—

¹Université de Sherbrooke, Sherbrooke, QC, Canada.

²Université Laval, Québec, QC, Canada.

Table 3. Oligonucleotide primer sequences used for PCR¹

Gene	Primer	Oligonucleotide sequence	PCR product (bp)
<i>sea</i>	SEA-F	CAGCATACTATATTGTTTAAAGGC	400
	SEA-R	CCTCTGAACCTTCCCATC	
<i>seb</i>	SEB-F	GTATGGTGGTGTAACTGAGCA	351
	SEB-R	TCAATCTTCACATCTTTAGAATCA	
<i>sec</i>	SEC-F	CTCAAGAACTAGACATAAAAGCTAGG	271
	SEC-R	TCAAAATCGGATTAAACATTATCC	
<i>sed</i>	SED-F	CTAGTTTGGTAATATCTCCTTTAAACG	319
	SED-R	TTAATGCTATATCTTATAGGGTAAACATC	
<i>seg</i>	SEG-F	AAGTAGACATTTTTGGCGTTCC	287
	SEG-R	AGAACCATCAAACCTCGTATAGC	
<i>selk</i>	SEK-F	TAGGTGTCTCTAATAATGCCA	293
	SEK-R	TAGATATTCGTTAGTAGCTG	
<i>sell</i>	SEL-F	TAACGGCGATGTAGGTCCAGG	383
	SEL-R	CATCTATTTCTTGTGCGGTAAC	
<i>selm</i>	SEM-F	GGATAATTGACAGTAACAG	379
	SEM-R	TCCTGCATTAATCCAGAAC	
<i>seln</i>	SEN-F	CATCATGCTTATACGGAGGAG	301
	SEN-R	CCCCTGAACCTTTTACGTT	
<i>selo</i>	SEO-F	TGTGTAAGAAGTCAAGTGTAG	214
	SEO-R	TCTTTAGAAATCGCTGATGA	
<i>selp</i>	SEP-F	TGATTTATTAGTAGACCTTGG	381
	SEP-R	ATAACCAACCGAATCACCAG	
<i>selq</i>	SEQ-F	TCAAGGAGTTAGTTCTGGAAATT	251
	SEQ-R	GCTTACCATTGACCCAGAGA	
<i>tst</i>	TST-F	AAGCCCTTTGTTGCTTGCG	447
	TST-R	ATCGAACTTTGGCCCATACTTT	

¹Sequences from Park et al. (2011).

results are in agreement with the study of Nemati et al. (2008) done with 102 bovine CNS isolated in Belgium. In that study, none of the SAg genes evaluated were found in their strains collection. However, as indicated above, other studies have reported the presence of enterotoxin and SAg genes in 54% of the bovine CNS isolated in Italy (40/74; Bertelloni et al., 2015), 53% in Poland (16/30; Fijalkowski et al., 2014), 47% in Brazil (61/128; Rall et al., 2014), and 31% in the United States (82/263; Park et al., 2011). Based on our results, the high prevalence observed in some studies might be attributable, at least in part, to nonspecific amplifications. Confirmation by other means (e.g., sequencing of the amplicons, detection of mRNA, or detection of the enterotoxins and SAg production; Wu et al., 2016) is therefore highly recommended.

This is, to the best of our knowledge, the first study done with bovine CNS in Canada. Although this pilot study was done with a relatively small selection of bacterial isolates and SAg genes, the absence of SAg suggests that CNS isolated in milk in Canadian dairy farms do not seem to represent a risk for public health.

ACKNOWLEDGMENTS

This work was supported by a New Initiative grant from Op+Lait Strategic Cluster (St-Hyacinthe, QC, Canada) to MJ and by a grant from the Dairy Research Cluster II (Dairy Farmers of Canada, Agriculture and Agri-Food Canada, Canadian Dairy Network, Canadian Dairy Commission) through the Canadian Bovine Mastitis and Milk Quality Research Network (St-Hyacinthe, QC, Canada) to MJ. CG is the recipient of an NSERC-CREATE in milk quality (St-Hyacinthe, QC, Canada) scholarship. AC and CG are the recipients of an Op+Lait Strategic Cluster scholarship. We have no conflicts of interest to declare.

REFERENCES

- Bertelloni, F., F. Fratini, V. Valentina Ebani, A. Galiero, B. Turchi, and D. Cerri. 2015. Detection of genes encoding for enterotoxins, TSST-1, and biofilm production in coagulase-negative staphylococci from bovine bulk tank milk. *Dairy Sci. Technol.* 95:341–352. <https://doi.org/10.1007/s13594-015-0214-9>.
- Cameron, M., H. W. Barkema, J. De Buck, S. De Vliegheer, M. Chaffer, J. Lewis, and G. P. Keefe. 2017. Identification of bovine-associated coagulase-negative staphylococci by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry using direct transfer protocol. *J. Dairy Sci.* 100:2137–2147. <https://doi.org/10.3168/jds.2016-12020>.
- Cavaillon, J.-M. 2018. Exotoxins and endotoxins: Inducers of inflammatory cytokines. *Toxicon* 149:45–53. <https://doi.org/10.1016/j.toxicon.2017.10.016>.
- Condas, L. A. Z., J. De Buck, D. B. Nobrega, D. A. Carson, S. Nausshad, S. De Vliegheer, R. N. Zadoks, J. R. Middleton, S. Dufour, J. P. Kastelic, and H. W. Barkema. 2017. Prevalence of non-aureus staphylococci species causing intramammary infections in Canadian dairy herds. *J. Dairy Sci.* 100:5592–5612. <https://doi.org/10.3168/jds.2016-12478>.
- Fijalkowski, K., M. Struk, J. Karakulska, A. Paszkowska, S. Giedry-Kalemba, H. Masiuk, D. Czernomysy-Furawicz, and P. Nawrotek. 2014. Comparative analysis of superantigen genes in *Staphylococcus xylosus* and *Staphylococcus aureus* isolates collected from a single mammary quarter of cows with mastitis. *J. Microbiol.* 52:366–372. <https://doi.org/10.1007/s12275-014-3436-2>.
- Fisher, L., M. Otto, and G. Y. C. Cheung. 2018. Basis of virulence in enterotoxin-mediated staphylococcal food poisoning. *Front. Microbiol.* 9:436. <https://doi.org/10.3389/fmicb.2018.00436>.
- Fry, P. R., J. R. Middleton, S. Dufour, J. Perry, D. Scholl, and I. Dohoo. 2014. Association of coagulase-negative staphylococcal species, mammary quarter milk somatic cell count, and persistence of intramammary infection in dairy cattle. *J. Dairy Sci.* 97:4876–4885. <https://doi.org/10.3168/jds.2013-7657>.
- Goetz, C., Y. D. N. Tremblay, D. Lamarche, A. Blondeau, A. M. Gaudreau, J. Labrie, F. Malouin, and M. Jacques. 2017. Coagulase-negative staphylococci species affect biofilm formation of other coagulase-negative and coagulase-positive staphylococci. *J. Dairy Sci.* 100:6454–6464. <https://doi.org/10.3168/jds.2017-12629>.
- Le Loir, Y., F. Baron, and M. Gautier. 2003. *Staphylococcus aureus* and food poisoning. *Genet. Mol. Res.* 2:63–76.
- Necidova, L., K. Bogdanovicova, D. Harustiakova, and K. Bartova. 2016. Pasteurization as a mean of inactivating staphylococcal enterotoxins A, B, and C in milk. *J. Dairy Sci.* 99:8638–8643. <https://doi.org/10.3168/jds.2016-11252>.
- Nemati, M., K. Hermans, D. Vancraeynest, S. De Vliegheer, O. C. Samponin, M. Baele, E. M. De Graaf, F. Pasmans, and F. Haesebrouck. 2008. Screening of bovine coagulase-negative staphylococci from milk for superantigen-encoding genes. *Vet. Rec.* 163:740–743.
- Oliveira, D., A. Borges, and M. Simoes. 2018. *Staphylococcus aureus* toxins and their molecular activity in infectious diseases. *Toxins (Basel)* 10:E252. <https://doi.org/10.3390/toxins10060252>.
- Park, J. Y., L. K. Fox, K. S. Seo, M. A. McGuire, Y. H. Park, F. R. Rurangirwa, W. M. Sischo, and G. A. Bohach. 2011. Detection of classical and newly described staphylococcal superantigen genes in coagulase-negative staphylococci isolated from bovine intramammary infections. *Vet. Microbiol.* 10:149–154. <https://doi.org/10.1016/j.vetmic.2010.06.021>.
- Podkowik, M., J. Y. Park, K. S. Seo, J. Bystron, and J. Bania. 2013. Enterotoxigenic potential of coagulase-negative staphylococci. *Int. J. Food Microbiol.* 163:34–40. <https://doi.org/10.1016/j.ijfoodmicro.2013.02.005>.
- Rall, V. L., E. S. Miranda, I. G. Castilho, C. H. Camargo, H. Langoni, F. F. Guimaraes, J. P. Araujo Junior., and A. Fernandez Junior.. 2014. Diversity of *Staphylococcus* species and prevalence of enterotoxin genes isolated from milk of healthy cows and cows with subclinical mastitis. *J. Dairy Sci.* 97:829–837. <https://doi.org/10.3168/jds.2013-7226>.
- Reyher, K. K., S. Dufour, H. W. Barkema, L. Des Côteaux, T. J. Devries, I. R. Dohoo, G. P. Keefe, J. P. Roy, and D. T. Scholl. 2011. The National Cohort of Dairy Farms—A data collection platform for mastitis research in Canada. *J. Dairy Sci.* 94:1616–1626. <https://doi.org/10.3168/jds.2010-3180>.
- Tremblay, Y. D., D. Lamarche, P. Chever, D. Haine, S. Messier, and M. Jacques. 2013. Characterization of the ability of coagulase-negative staphylococci isolated from the milk of Canadian farms to form biofilms. *J. Dairy Sci.* 96:234–246. <https://doi.org/10.3168/jds.2012-5795>.
- Tuffs, S. W., S. M. Mansour Haeryfar, and J. K. McCormick. 2018. Manipulation of innate and adaptive immunity by staphylococcal superantigens. *Pathogens* 7:E53. <https://doi.org/10.3390/pathogens7020053>.
- Wu, S., N. Duan, H. Gu, L. Hao, H. Ye, W. Gong, and Z. Wang. 2016. A review of the methods for detection of *Staphylococcus aureus* enterotoxins. *Toxins (Basel)* 8:176. <https://doi.org/10.3390/toxins8070176>.