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The Analysis of Milk Components and Pathogenic Bacteria Isolated from Bovine Raw Milk in Korea

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ABSTRACT

Bovine mastitis can be diagnosed by abnormalities in milk components and somatic cell count (SCC), as well as by clinical signs. We examined raw milk in Korea by analyzing SCC, milk urea nitrogen (MUN), and the percentages of milk components (milk fat, protein, and lactose). The associations between SCC or MUN and other milk components were investigated, as well as the relationships between the bacterial species isolated from milk. Somatic cell counts, MUN, and the percentages of milk fat, protein, and lactose were analyzed in 30,019 raw milk samples collected from 2003 to 2006. The regression coefficients of natural logarithmic-transformed SCC (SCCt) on milk fat (–0.0149), lactose (–0.8910), and MUN (–0.0096), and those of MUN on milk fat (–0.3125), protein (–0.8012), and SCCt (–0.0671) were negative, whereas the regression coefficient of SCCt on protein was positive (0.3023). When the data were categorized by the presence or absence of bacterial infection in raw milk, SCCt was negatively associated with milk fat (–0.0172), protein (–0.2693), and lactose (–0.4108). The SCCt values were significantly affected by bacterial species. In particular, 104 milk samples infected with *Staphylococcus aureus* had the highest SCCt (1.67) compared with milk containing other mastitis-causing bacteria: coagulase-negative staphylococci (n = 755, 1.50), coagulase-positive staphylococci (except *Staphylococcus aureus*; n = 77, 1.59), *Streptococcus* spp. (*Streptococcus dysgalactiae*, n = 37; *Streptococcus uberis*, n = 12, 0.83), *Enterococcus* spp. (n = 46, 1.04), *Escherichia coli* (n = 705, 1.56), *Pseudomonas* spp. (n = 456, 1.59), and yeast (n = 189, 1.52). These results show that high SCC and MUN negatively affect milk components and that a statistical approach associating SCC, MUN, and milk components by bacterial infection can explain the patterns among them. Bacterial species present in raw milk are an important influence on SCC in Korea.

Key words: bovine mastitis, somatic cell count, milk urea nitrogen, *Staphylococcus aureus*

INTRODUCTION

Mastitis is an inflammation of the mammary glands of dairy cows that can be caused by physical or chemical agents, with the majority of cases caused by bacterial infection. Mastitis is the most common and expensive disease affecting the dairy industry worldwide (Harmon, 1994; Quinn et al., 1994; Moussaoui et al., 2004). In Korea, there are approximately 8,000 dairy farms and 472,000 cows, yielding an average of 177,770,000 kg of raw milk per year. The degree of self-sufficiency of milk produced in Korea is approximately 70% (Korea Dairy Committee, 2007), so managing and preventing bovine mastitis is an inevitable task.

One indicator of bacterial infection of the mammary glands is an increase in SCC, which has been used to monitor mastitis in dairy cows (O'Brien et al., 2001). Milk urea nitrogen is an indicator of protein utilization (Jonker et al., 1999) and can be increased by excessive feeding of protein (Broderick and Clayton, 1997). Therefore, SCC and MUN have become good management tools for predicting and diagnosing mastitis and for monitoring the use of protein and the improvement in milk quality (Harmon, 1994; Jonker et al., 2002).

Elevated SCC has been associated with a decrease in the percentages of lactose and fat in milk (Harmon, 1994). Mammary epithelial cells can be damaged by bacteria, resulting in a reduced ability to synthesize milk components. Moreover, MUN has been inversely associated with percentages of milk protein and fat and with SCC (Eicher et al., 1999; Godden et al., 2001; Johnson and Young, 2003).

More than 130 microorganisms are related to bovine mastitis, with mastitis-causing bacteria broadly classi-

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fied as contagious or environmental pathogens (Watts, 1988; Quinn et al., 1994). Contagious pathogens such as *Staphylococcus aureus* and *Streptococcus agalactiae* can be transmitted from cow to cow (Bradley, 2002), whereas environmental pathogens, such as *Streptococcus dysgalactiae*, *Streptococcus uberis*, *Enterococcus* spp., CNS, and gram-negative enteric bacilli (*Pseudomonas* spp., *Escherichia coli*) can be transmitted during milking from the contaminated environment (Watts, 1988).

The objectives of this study were to examine and predict the quality of raw milk by analyzing SCC, MUN, and milk components of Holstein cows in Korea. We investigated the associations among SCC, MUN, and milk components based on the presence of bacteria and the bacterial species isolated from these raw milk samples.

MATERIALS AND METHODS

Milk Sampling and Analysis

From March 2003 to March 2006, a total of 30,019 bovine raw milk samples were randomly collected from 390 farms in 9 provinces in Korea. Teat ends were cleaned with 4% chlorhexidine before sampling. The first few milliliters of milk were discarded, and then quantities of 20 to 50 mL of milk were collected aseptically into sterile vials. Milk samples were transported on ice to the laboratory and kept at 4°C until bacteriological assays and analysis of SCC, MUN, and milk components. Somatic cell counts and MUN were determined by Somacount 150 and Chemspec 150 instruments (Bentley Instruments Inc., Chaska, MN), respectively. The percentages of milk components, including milk fat, milk protein, and lactose, were analyzed by using a Bentley 150 instrument (Bentley Instruments Inc.).

Somatic cell count values were sorted into 5 categories: $<200 \times 10^3$ cells/mL (grade 1); 200 to 349×10^3 cells/mL (grade 2); 350 to 499×10^3 cells/mL (grade 3); 500 to 750×10^3 cells/mL (grade 4); and $>751 \times 10^3$ cells/mL (grade 5). Milk urea nitrogen was sorted in increments of 2 mg/dL; samples with MUN ≤6 mg/dL were grouped into one category, and those with MUN ≥24 mg/dL into another category (Johnson and Young, 2003).

Isolation and Identification of Bacteria

Ten microliters of each milk sample was streaked onto 5% sheep blood agar plates (Promed, Sungnam, Gyeonggi, Korea) and incubated at 37°C for 24 h. Colonies were initially assessed by their morphology and hemolysis patterns, followed by gram staining and motility tests. Biochemical tests, specifically, catalase, oxidase, coagulase, growth in 6.5 or 10% NaCl, esculin hydrolysis, carbohydrate (glucose, mannitol, ribose, sorbitol, and trehalose) fermentation tests, biochemical reaction on MacConkey agar (Becton Dickinson and Co., Sparks, MD), indole production, Lys decarboxylation, urease production, and citrate utilization tests, were performed as required.

Determination of the Identified Bacteria by PCR

The identification of *Staph. aureus*, *Strep. dysgalactiae*, *Strep. uberis*, and *Enterococcus* spp. were further confirmed by PCR by using species-specific primers (Brakstad et al., 1992; Forsman et al., 1997; Martineau et al., 1998; Ke et al., 1999; Table 1). Chromosomal DNA was extracted by the guanidine thiocyanate method. Briefly, after culturing in 5% sheep blood agar, a single colony was inoculated into 3 mL of trypticase soy broth (Becton Dickinson and Co.) and incubated at 37°C for 24 h. The broth culture was centrifuged for 10 min at $6,000 \times g$ in a 1.5-mL microcentrifuge tube, and the pellet was resuspended in 200 μL of enzymatic lysis buffer [10 m*M* Tris-HCl, 1 m*M* EDTA, pH 8.0, 1.2% Triton X-100, and 18 μg of lysozyme (Sigma-Aldrich, St. Louis, MO)]. In cases in which streptococcal isolates were suspected, 2.5 U/mL of mutanolysin (Sigma-Aldrich) was added. For *Staph. aureus*, the pellet was resuspended in 1 mL of TE buffer (10 m*M* Tris-HCl and 1 m*M* EDTA, pH 8.0) containing 100 U/mL of lysostaphin (Sigma-Aldrich), to which 40 μL of diatomaceous earth suspension [10 g of diatomaceous earth (Sigma-Aldrich), 50 mL of tertiary distilled water, and 500 μL of 32% HCl (wt/vol)] was added. The suspension was mixed by vortexing, incubated at room temperature for 10 min, and centrifuged for 2 min at $20,000 \times g$. The pellet was washed with a second washing buffer [120 g of guanidine thiocyanate (Amresco, Solon, OH) and 100 mL of 0.1 *M* Tris HCl (pH 6.4)], serially washed with 70% ethanol and 100% acetone, and incubated at 56°C for 10 min. The DNA was eluted in 200 μL of Tris-EDTA buffer and incubated at 56°C for 10 min. The supernatant was transferred to a new tube after centrifugation for 2 min at $20,000 \times g$.

Polymerase chain reaction was performed in a Mastercycler Gradient Thermal Cycler (Eppendorf Mastercycler Gradient, Hamburg, Germany), and all reagents were purchased from Takara Bio Inc. (Otsu, Shiga, Japan). Each PCR reaction mixture was made up to a final volume of 20 μL, consisting of 13.7 μL of distilled water, 0.4 μL of each primer (10 pmol/μL; Table 1), 1.6 μL of 2.5 m*M* dNTP, 1.2 μL of 25 m*M* $MgCl_2$, 2 μL of 10× buffer, 5 U of *Taq* polymerase (0.1 μL),