

that Stxs expression was significantly decreased ~2.98-folds in the EC medium without lactose, while it was slightly increased in the EC medium without bile salts, compared to the original EC medium. Consistently, the transcription expression of the *stx 1 & 2* genes was drastically increased in cells grown in LB medium supplemented with lactose, compared to cells in LB medium, indicating that lactose is an environmental trigger of Stxs expression.

Conclusions: The results imply that lactose functions as an inducer of the expression of Stxs in EHEC O157:H7. Further study is necessary to determine the molecular mechanism behind the lactose-mediated induction of Stxs in this microorganism.

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P-059

Antimicrobial Effect of a Novel Peptide-peptide Nucleic Acid Antisensing the Cytidine Monophosphate kinase of *Staphylococcus aureus*

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Introduction: *Staphylococcus aureus* can easily acquire bacterial resistance to several antimicrobial drugs and thus, novel antibiotics and/or non-antibiotic based anti-infective strategies need to be developed for the intervention of notorious methicillin- or multidrug-resistant *S. aureus*.

Materials and Methods: Based on the genome sequence of *S. aureus* N315, a set of PNA conjugates with a bacterial penetration peptide (KFF)₃K-L- were synthesized to antisense the seven potentially essential genes (*cmk*, *deoD*, *ligA*, *smpB*, *glmU*, *pyrH*, and *ftsA*) and further evaluated for their antibacterial properties *in vitro* as well as *in vivo*.

Results: Our experimental analyses demonstrated that two peptide conjugated-PNAs (P-PNAs) targeting either *cmk* or *deoD* genes, Pjyh-cmk1 and Pjyh-deoD1, had the strongest growth inhibitory effects against *S. aureus* ATCC 29740 (a bovine mastitic milk isolate) in a dose-dependent manner. Using a translational fusion system with the *lacZ* reporter gene, such an inhibitory activity in bacterial growth was due to the antisense effect against the target genes rather than the antigene effect. *In vivo* application of Pjyh-cmk1 resulted in the significant reduction of bacterial loads in the intra-

peritoneally infected mice with a sublethal dose of *S. aureus* at 20 hrs post-infection. Moreover, Pjyh-cmk1 could dramatically increase the survival rate of the mice lactated after intramammary infection.

Conclusions: Our characterization of Pjyh-cmk1 demonstrated the bactericidal activity against *S. aureus*, an important zoonotic bacterial pathogen, as well as *in vivo* effectiveness. This is another demonstration implying possible application of the antisense P-PNA as an alternative anti-infective agent.

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P-060

Prevalence, Antibiotic Resistance, and Virulence Features of Shiga Toxin-producing *Escherichia coli* Isolates from the Retail Meats and Meat by-products in Korea

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Introduction: Healthy cattle are the most important animal reservoir associated with human infection by Shiga toxin-producing *Escherichia coli* (STEC). So, consumption of raw or undercooked meat is the most common route of transmission of STEC. In this study, we isolated and identified STEC from retail meats or meat by-products in Korea. Their virulence potentials and clonality were also examined.

Materials and Methods: All the samples from retail meats or meat by-products were applied to a standard STEC isolation procedure. STEC isolates were serotyped and screened by PCR to determine their virulence potentials (*stx1*, *stx2*, *saa*, *eae*, *ehxA*, *tir*, and *espB*). The functionality of the *rpoS* gene product was examined by Western blot. Plasmid profiling, swimming motility, antibiogram, and PFGE were further analyzed to characterize the STEC isolates.

Results: A total of 299 (68.89%) *E. coli* were isolated from the 174 retail meats and the 106 meat by-products. Among them, the only 7 (1.61%) *E. coli* isolates possessed the *stx1* or *2* genes. Our experimental analyses revealed that they belonged into the 4 different serotypes including O91:H14, O121:H10, O91:H21, and Ont:H20. Moreover, the two STEC O91:H14 isolates were non-motile although they had a functional copy of the *fliC* gene. None of them carried the *eae*, *tir*, and *espB* genes, indicating the absence of the LEE pathogenicity island in these STEC isolates. In contrast, they

all possessed the *ecpA* gene with or without the *saa* gene, previously known as an adhesin for LEE-negative STEC isolates. The plasmid profiling demonstrated that the 4 STEC isolates harbor the 60-megadalton virulence plasmid, which was confirmed by the PCR against the *ehxA* gene. The two STEC O121:H10 isolates could not produce the *rpoS* gene product for unknown reasons although its biological significance is unclear. Notably, our STEC isolates were highly susceptible to the antimicrobials evaluated and one clonal set was identified by PFGE analysis.

Conclusions: Our results demonstrated the recent prevalence of STEC among retail meats and meat by-products in Korea as well as their genetic properties. Further study might be needed to elucidate the *in vivo* virulence of the STEC isolates and to define geographical difference between our STEC and those from other countries.

References

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P-061

Characterization of Blood Circulating CD209 (DC-SIGN) Expressing Cells in Bovine using Flow Cytometry

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Introduction: Dendritic cells (DC) play a central role in tailoring the immune response to pathogens. CD209 (DC-specific ICAM3 grabbing nonintegrin, DCSIGN) is a C-type lectin receptor expressed on DC that plays a critical role on DC function and pathogen recognition. It facilitates DC migration to peripheral tissues and local lymph nodes and mediates T cell activation by binding ICAM-2 (CD102) and ICAM-3 (CD50). The absence of monoclonal antibody (mAb) to bovine CD209 has limited the ability to characterize the phenotype and function of DC in cattle. To address this issue we developed and used a mAb to CD209 to characterize the phenotype of CD209-expressing cells in bovine blood using flow cytometry.

Materials and Methods: Bovine CD209 recombinant protein was expressed and purified using pET30a expression system (Novagen). The purified recombinant protein was used for immunization of BALB/c mice to produce a mAb for bovine CD209. The established mAb (209MD26A) was used to detect and characterize blood circulating CD209 positive cells using flow cytometric analysis.

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characterize blood circulating CD209 positive cells using flow cytometric analysis.

Results: About 1 – 2.5 % of bovine PBMC subset expressed CD209. This subset highly expressed the molecules functionally associated with antigen presentation, MHC II, CD40, CD80, and CD86. In addition, the lineage markers expressed on myeloid DC, CD11b, CD11c, and CD172a, were also highly expressed on these cells, indicating this subset contains myeloid DC.

Conclusions: A mAb to bovine CD209 (209MD26A) was successfully developed in the current study. The mAb detects a population in PBMC corresponding to human CD209⁺DC. The availability of this mAb provides opportunity for further characterization of DC in ruminants.

P-062

Effectiveness of Brucellosis Eradication Program in the Republic of Korea during Decline Period

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Introduction: A national surveillance program of bovine brucellosis has been performed in Korea since 1960s. Due to strong implementation based on test-and-slaughter policy, both annual numbers of outbreak farms and reactor animals detected were in decreasing since year 2008. This study evaluated economical benefit from Korean's efforts to eradicate bovine brucellosis.

Materials and Methods: Governmental statistics on livestock and brucellosis were extracted from web-based database. Four different deterministic models were established using Vensim PLE 6.3 with susceptible to infectious structure. One transient model was for brucellosis in cattle and the second was for human. These two models were linked through cattle to human transmission. In addition, two separate models were developed for beef and dairy cattle, respectively.

Results: Incremental savings, estimated by subtracting actual number of reactors from the number of cattle reactors predicted at the endemic stable transmission status, was 96,346 cattle cases between 2006 to 2013. The incremental saving of cattle cases were predicted to 154,361 during 2014 to 2020, and 281,324 when five more years are included. The total cost averted for the period of 2006 to 2013 was 327,927 million for cattle only while it increased to 328,206 million with human cases.

Conclusions: The national program for eradicating bovine brucellosis has been well performed. It contributed to the decrease both number of cases in cattle and human. However, with the test-and-slaughter policy, benefits on financial aspects won't be expected in near future. Concerning eradicating program of bovine brucellosis, macroeconomic effects is greater than microeconomic ones.