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Evaluation of the probiotic properties of lactic acid bacteria isolated from chickens and ducks

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Introduction: Lactic acid bacteria(LAB) have been suggested beneficial for human and animal hosts and been used as effective components of many probiotics. Because there are substantial differences between different animal species in intestinal environments, potential probiotics need to be selected from the same host species where they are intended to be used. Therefore, we isolated LAB from intestinal contents of chickens and ducks and characterized isolates to select candidate probiotic strains for poultry.

Materials and Methods: Cecal contents from healthy chickens (n=40) and ducks (n=24) were plated on selective medium. LAB-suspect colonies were selected and characterized by 16S rDNA sequence analysis. LAB identified was tested for their *in vitro* probiotic traits such as enzyme activity, antibiotic resistance, antimicrobial activity, exopolysaccharide (EPS) production, and acid and bile tolerance.

Results: A total of 29 strains of LAB belonging to 11 species were identified. Four *Lactobacillus salivarius*, 1 *Lactobacillus sakei*, 1 *Lactobacillus coryniformis* subsp. *torquens*, and 1 *Lactobacillus crispatus* showed no undesirable enzymatic activity. One *L. coryniformis* subsp. *torquens*, 1 *Lactobacillus animalis*, 2 *Streptococcus alactolyticus* were resistant to kanamycin and streptomycin. Two *L. salivarius* showed the best antimicrobial activity. The amount of EPS produced ranged from 0 and 44.63 mg/ml. All 29 strains were tolerant to 0.3% w/v whereas only 15 strains showed tolerance at pH2.5.

Conclusions: Overall, a *L. salivarius*strain isolated from duck fulfilled the principal requirements of a qualified probiotic. This strain can be a reliable candidate for further validation studies in chickens and ducks.

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Application of polymerase chain reaction assay for simultaneous detection of *Clostridium chauvoei* and *Clostridium septicum*

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Introduction: *Clostridium chauvoei* and *Clostridium septicum* of pathogenic clostridia are acute infections with serious symptom and high rate of mortality. Cattle, sheep, goats and other herbivore species are susceptible to blackleg and malignant edema. For rapid diagnostic of these clostridia, the 16S-23S rRNA spacer region was applied for differentiation of blackleg causing *C. chauvoei* and *C. septicum*, a phylogenetically related bacterium responsible for malignant edema. In this study, the designed primer sequences were tested to confirm sensitivity and specificity of *C. chauvoei* and *C. septicum* by PCR (polymerase chain reaction) assay.

Materials and Methods: In order to confirm the specificity of *C. chauvoei* and *C. septicum* by PCR assay. 16S-23S rRNA spacer region of *C. chauvoei* (522 bp) and *C. septicum* (594 bp) used for amplification. Also *Clostridium dificille*, *E.coli*, *Salmonella* and *Bacillus anthracis* were tested with these samples. To identify the sensitivity, PCR was performed by 10-fold dilution of the DNA samples (*C. chauvoei* and *C. septicum*).

Results: *C. chauvoei* and *C. septicum* samples were detected in all of each strain. These products were not confirmed contamination and additional samples (*C. dificille*, *E.coli*, *Salmonella* and *Bacillus anthracis*). Analytical sensitivity of PCR assay for the 16S-23S rRNA gene was showed sequential results depending on concentration.

Conclusions: In conclusion, the proposed PCR assay combines all essential features of a diagnostic tool to differentiate *C. chauvoei* and *C. septicum*. Further testing of a large number of field specimens will be performed in the future.

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P-030

Expression of the heavy-chain receptor binding domain of *Clostridiumbotulinum* neurotoxin type B for recombinant vaccine candidate

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Introduction: *Clostridiumbotulinum* produced botulinum toxins (BoNTs) that inhibited the release ofacetylcholine at the neuromuscular junctions and synapses. The BoNTs consisted of50-kDa light chain (LC) and 100-kDa heavy chain (HC), and they were classifiedinto eight neurotoxin types (A to H). In particular, HC had two domains thatplayed a role in toxicity via translocation (HCT) and receptor binding (HCR).BoNT/B has been reported to critically affect cattle and horses. Severalcountries including Korea had

been used crude proteins of *Clostridium botulinum* for the toxoid vaccine. Unfortunately, thisapproach had lots of problems such as safety and manufacturing difficulty. Theaim of this study was to express and purify the recombinant HCR protein ofBoNT/B.

Materials and Methods: The HCR(50-kDa) of *Clostridium botulinum*neurotoxin type B was chosen as vaccine candidate and the PCR with thecomplementary primers was performed to amplify HCR gene. The PCR product wassub-cloned into TA- and pET28a-vector, and transformed in *Escherichia (E.) coli* BL21 expression host. Recombinant HCR proteinwas then over-expressed and it was purified by Nickel-based affinitychromatography. The purity of HCR was confirmed by SDS-PAGE and western blotwas also used to analysis of HCR protein.

Results: The results showing in this study demonstrated thatrecombinant HCR protein was successfully cloned and expressed in *E. coli* BL21, and the His-taggedrecombinant HCR protein was purely obtained by Ni^{2+} affinitycolumns. In addition, we established an optimal experimental condition ofpurification protocol for recombinant HCR protein by SDS-PAGE and western blotanalysis.

Conclusions: In this study, recombinant HCR protein was successfully over-expressed in bacterial system,and the recombinant HCR was purified. Further research will be needed toevaluate the purified HCR protein with challenge of BoNT/B in vaccinated mouse.

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Analysis of percutaneous absorption rates of propoxur using in vitro micro-pig dermal tissue model.

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Introduction: Propoxur is a widely used pesticide, which is approved to be used on animals in Korea. Recently, chicken eggs were found to contain pesticides in Europe and Asian country including South Korea. Although the pesticides have advantage of reducing ticks on laying hens, they can be a serious risk factor to the health of the food consumers due to the pesticides residues in eggs. Since there is a lack of previous data for the percutaneous absorption rate of propoxur in food-producing animals, we intended to measure in vitro dermal absorption rate of propoxur in food producing animals for regulation's purpose.

Materials and Methods: Skin slices of micro-pig were purchased from MEDIKINETICS Co. Inc. 2.5*2.5*500μm of franz cell membranes were used in this study. The integrity of excised skin was examined morphologically with hematoxylin-Eosin (H&E) staining. 250 ppm of propoxur was prepared by dissolving in methanol. 10 μL of propoxur solutions were applied to the skins mounted on franz diffusion cells. PBS solutions were taken after 1, 2, 4, 8, 12, 24, 48 and 72. Dermal tissues, tape strips, washing solutions and washing swabs were taken for analysis. All samples were prepared and extracted using modified QuEChERS methods. Analysis of propoxur was perfomed using LC-MS/MS (Shimatsu Shimadzu Nexera X2 / Absciex QTRAP 6500).

Results: The integrity and thickness of the franz cell membranes showed appropriate for this study. The absorbed propoxur was mainly present in the receptor in 72 hours of incubation. Total absorption rate was 70.4±7.2% of applied dose which was found 69.8% in receptor fluids and 0.6% in dermal tissues. 0.27% of applied propoxur did not penetrate into the skin and washed out in 72 hours. The sum amount recovered in all analyzed samples was 72.8±7.3% in this study.

Conclusions: In this study, propoxur showed rapid absorption through the pig skins. These results could support to drafting regulations on food safety of veterinary drugs, such as establishment of withdrawal period for the pesticide.

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P-032

Beneficial effects and current situation of animal assisted education

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Introduction: This article provides a review of research published since 1990 on the beneficial effects and current situation of animal assisted education.

Materials and Methods: Animal Assisted Education (AAE) is goal-directed interventions designed to promote improvement in cognitive functioning of the person(s) involved and in which a specially trained dog-handler team is an integral part of the educational process.