400 Development of an anaphylactic model of buckwheat using B-type CpG oligodeoxynucleotides from lactic acid bacteria. Yoshinari Yamamoto*[1], Suguru Shigemori[1,2], Kazushi Oshiro[3], Pengfei Wang[1], Yeqin Wang[1], Takashi Sato[4], and Takeshi Shimosato[1,5], *[1]Interdisciplinary Graduate School of Science and Technology, Shinshu University, Kamiina, Nagano, Japan, [2]Research Fellow of the Japan Society for the Promotion of Science (JSPS), Chiyoda, Tokyo, Japan, [3]Graduate School of Agriculture, Shinshu University, Kamiina, Nagano, Japan, [4]Graduate School of Medicine, Yokohama City University, Yokohama, Kanagawa, Japan, [5]Institute for Biomedical Sciences, Shinshu University, Kamiina, Nagano, Japan.*

Anaphylaxis is an acute systemic allergic reaction (severe type I hypersensitivity) to a specific allergen and occurs in human and other mammals. Food anaphylaxis is most frequently triggered by peanut, buckwheat, milk, egg, and wheat allergens. Anaphylactic mouse models against ovalbumin and peanut allergens have been established by repeated exposure to small amounts of allergen with adjuvant via subcutaneous (s.c.) and intraperitoneal (i.p.) routes. When immunity is established, the animals are challenged with a large quantity of allergens via an i.v. route to induce anaphylaxis. However, i.v. sensitization is a relatively complicated procedure, and establishing an anaphylactic model requires considerable time. In addition, the i.p. method for allergen delivery is simpler than i.v. routes, has lower risk for failure, and can shorten sensitization duration. Here, we attempted to develop a simple anaphylactic shock model by exposing mice to a B-type CpG-oligodeoxynucleotide (ODN), termed MsST, which had the immune ability to induce IL-33 and IFN-γ production from the *lac* Z gene of *Streptococcus thermophilus* ATCC19258 with allergen via an i.p. route. After the allergen challenge for inducing anaphylactic shock, IgG_{2a}-production and IFN-γ-positive cells were markedly increased in mice that received B-type CpG-ODN. In contrast, IL-33-positive cells in the model mice were only slightly increased. Generally, IFN-γ supports Th1 cell responses that suppress allergic disease. However, it was previously reported that IFN-γ enhances platelet-activating factor, which is a bioactive lipid mediator that plays a role in the onset of inflammatory diseases such as asthma, anaphylaxis and atherosclerosis, and is involved in the generation of $CD11b^+$ and $CD11c^+$ cells. The anaphylactic shock model described here is expected to contribute to the development of anaphylaxis treatment strategies.

Key Words: anaphylactic model, buckwheat, B-type CpG-oligodeoxynucleotide (ODN)

401 Construction of genetically modified *Lactococcus lactis* expressing buckwheat protein Fagag1 with strong allergenicity. Suguru Shigemori*[1,2], Yoshinari Yamamoto[1], Kazushi Oshiro[3], Pengfei Wang[1], Yeqin Wang[1], Takashi Sato[4], and Takeshi Shimosato[1,5], *[1]Interdisciplinary Graduate School of Science and Technology, Shinshu University, Kamiina, Nagano, Japan, [2]Research Fellow of the Japan Society for the Promotion of Science (JSPS), Chiyoda, Tokyo, Japan, [3]Graduate School of Agriculture, Shinshu University, Kamiina, Nagano, Japan, [4]Graduate School of Medicine, Yokohama City University, Yokohama, Kanagawa, Japan, [5]Institute for Biomedical Sciences, Shinshu University, Kamiina, Nagano, Japan.*

Buckwheat (*Fagopyrum esculentum*) is consumed as a pseudocereal in Asia and Western countries, and is recognized as a functional food. However, several proteins from buckwheat have strong allergenicity and cause severe symptoms, such as an anaphylaxis, in hypersensitive patients. Here, we engineered strains of *Lactococcus lactis* NZ9000 (NZ9000) that express Fagag1, a major allergenic storage protein of buckwheat, fused with or without green fluorescent protein (GFP). Codon-optimized sequences of Fagag1 and GFP-Fagag1 were individually cloned into the *L. lactis* expression vector pNSH, containing a nisin-inducible promoter and 6x histidine-tag sequence. The resulting plasmids were separately introduced into NZ9000, and nisin-dependent expression of recombinant Fagag1 (rFagag1) and rGFP-Fagag1 was confirmed by Western blotting and confocal laser scanning microscopy. rFagag1 and rGFP-Fagag1 were individually purified by immobilized metal-ion affinity chromatography techniques, and the allergenicity of the purified proteins was then evaluated in *in vitro* cultures of splenocytes isolated from buckwheat crude protein-immunized mice. Treatment of splenocytes with rFagag1 markedly induced the mRNA expression of interleukin (IL)-4, IL-13, and IL-17F, which are known mediators of allergic inflammation. Similar expression levels of IL-4 and IL-17F mRNA were observed in the splenocytes stimulated with purified rGFP-Fagag1; however, the increases from baseline were significantly lower than those observed in rFagag1-treated cells. Recent evidence suggests that the mucosal (i.e., oral or intranasal) application of lactic acid bacteria genetically modified to produce allergen is a promising strategy for allergy therapy. Therefore, our present results suggest that NZ9000 expressing immunoreactive rFagag1 or rGFP-Fagag1 may be a powerful candidate for the prevention and therapy of buckwheat allergy. However, the future application of prophylactic and therapeutic strategies based on NZ9000 strains first requires a clear demonstration of efficacy in in vivo trials.

Key Words: buckwheat, *Lactococcus lactis*, food allergy

402 Production of recombinant β-lactoglobulin in *Lactococcus lactis* and generation of a bioactive peptide with incretin-inactivation activity. Kazushi Oshiro*[1], Suguru Shigemori[2,3], Yoshinari Yamamoto[2], Pengfei Wang[2], Yeqin Wang[2], Takashi Sato[4], and Takeshi Shimosato[2,5], *[1]Graduate School of Agriculture, Shinshu University, Kamiina, Nagano, Japan, [2]Interdisciplinary Graduate School of Science and Technology, Shinshu University, Kamiina, Nagano, Japan, [3]Research Fellow of the Japan Society for the Promotion of Science (JSPS), Chiyoda, Tokyo, Japan, [4]Graduate School of Medicine, Yokohama City University, Yokohama, Kanagawa, Japan, [5]Institute for Biomedical Sciences, Shinshu University, Kamiina, Nagano, Japan.*

β-Lactoglobulin (BLG) is the most abundant protein in cow's milk whey. Peptides prepared from BLG digested with the gastrointestinal (GI) proteases pepsin and trypsin strongly inhibit dipeptidyl peptidase-IV (DPP-IV) activity. DPP-IV is a ubiquitously expressed serine protease that cleaves N-terminal dipeptides from incretins, which are insulinotropic GI hormones. Recently, genetically modified lactic acid bacteria have emerged as efficient cell factories for the production and safe delivery of heterologous proteins to the GI tract. Here, we engineered a strain of *Lactococcus lactis* to secrete recombinant BLG (rBLG) and evaluated the DPP-IV-inhibiting activity of trypsin-digested rBLG. To construct rBLG-producing *L. lactis*, a codon-optimized bovine BLG gene was inserted into the lactococcal secretion vector pNZ8148#2:SEC, containing a nisin-inducible promoter and signal peptide sequence from USP45 protein. The constructed rBLG secretion vector was transformed into *L. lactis* NZ9000 (NZ9000) as a host strain for gene expression. The

secretion of rBLG by NZ9000 was confirmed by Western blotting. The DPP-IV-inhibitory activity of trypsin-digested rBLG was examined by comparative analysis with trypsin-digested commercial BLG (cBLG) using a DPP-IV Drug Discovery Kit. Results from the assay revealed that the inhibitory activity of trypsin-digested rBLG against DPP-IV was similar to that of cBLG. In conclusion, we successfully engineered a strain of NZ9000 that efficiently secretes rBLG, and clearly demonstrated that trypsin-digested rBLG has strong inhibitory activity against DPP-IV enzyme. In future studies, we plan to evaluate the potential of rBLG-secreting NZ9000 as a therapeutic agent for type 2 diabetes.

Key Words: β-lactoglobulin, *Lactococcus lactis*, dipeptidyl peptidase-IV

403 Sodium chloride induced stress responses in dairy probiotic bacteria. Akanksha Gandhi* and Nagendra P. Shah, *The University of Hong Kong, Hong Kong.*

The study focused on possible mechanisms of salt stress injury and responses of selected probiotic bacteria to salt stress. The effects of varying NaCl/KCl concentrations on viability, membrane integrity and metabolic activity of *Lactobacillus acidophilus*, *Lactobacillus casei* and *Bifidobacterium longum* were studied using flow cytometry. Furthermore, the changes in surface functional groups, morphology and membrane fatty acid composition of these bacteria were investigated using Fourier-transform infrared (FTIR) spectroscopy, transmission electron microscopy and gas chromatography. The effect of NaCl/KCl stress on the in vitro adhesion ability of stressed bacteria to Caco-2 cells was also evaluated. Cell viability as evaluated using conventional culture technique was compared with the findings from flow cytometric analysis on metabolic activities of the cells and it was revealed that there was a correlation between culturability and dye extrusion ability of *Lb. casei* and *B. longum*. However, a certain population of *Lb. acidophilus* was viable as per the plate count method but the efflux activity was compromised. The metabolic activity of *Lb. casei* was found to be highest among the 3-probiotic bacteria. The FTIR spectral analysis also highlighted the shifts that occurred mainly in the amide regions upon increasing the NaCl concentration. Significant changes in the morphology of all bacteria were observed at higher salt concentrations. Shrinkage of the cytoplasmic content and irregularities in cell wall on exposure to high NaCl concentrations (10% w/v) were observed in all bacteria. Membrane fatty acid composition was affected by salt stress, and the ratio of unsaturated to saturated fatty acids was altered on exposure to NaCl stress. Adhesion ability of stressed bacteria to Caco-2 cell lines was also reduced at higher NaCl concentrations (10% w/v). Comparing the responses among the selected bacteria, *Lb. casei* appeared to be most robust to NaCl stress. Overall, the study revealed the impact of salt stress on membrane characteristics and adhesion capability of selected probiotic bacteria.

Key Words: sodium chloride, lactic acid bacteria, cell membrane

404 Potential role of *Bacillus* strains isolated from the dairy environment as defect-causing organisms in yogurt. Dipakkumar Mehta*[1], Ashraf Hassan[2], Brandon Nelson[2], and Hasmukh Patel[1], *[1]South Dakota State University, Brookings, SD, [2]Daisy Brand, Garland, TX.*

Bacillus spp. can cause spoilage of milk and dairy products due to their ability to produce heat resistant enzymes and spores. Milk proteins, fat, and stabilizers (such as gelatin, amylopectin (AP), xanthan, and pectin) play an important role in structure formation and stability of yogurt. Possible sources of *Bacillus* spp. in these products include raw milk, ingredients such as dairy powders, and processing equipment. The objective of this study was to test the proteolytic, lipolytic, and phospholipase activities as well as stabilizer-degrading characteristics of 25 *Bacillus* strains isolated from the dairy environment. Proteolysis, lipolysis, and phospholipase activity were tested using skim-milk agar, spirit blue agar, and lecithin agar, respectively. A basic agar medium containing AP, xanthan, or pectin and gelatin agar were used to study the stabilizer-degradation activities of the various *Bacillus* strains. All experiments were conducted in triplicate at 42°C and under mesophilic conditions. Many of the tested *Bacillus* strains were highly proteolytic and could degrade various stabilizers (AP, pectin, xanthan and gelatin). It was found that the majority (92%) of *Bacillus* strains were able to degrade AP while only 20% showed lipolytic activities. All proteolytic strains showed gelatin degradation activities. The xanthan, phospholipid, and pectin degradation activities were found in 68%, 40% and 24% *Bacillus* strains, respectively. Generally, *Bacillus* spp. exhibited higher activities at 42°C than at mesophilic temperatures. Based on these results, it can be concluded that the order of susceptibility of the different tested components to *Bacillus* strains was AP > protein = gelatin > xanthan > phospholipids > pectin > fat, suggesting their ability to induce texture defects in yogurt.

Key Words: *Bacillus,* yogurt, stabilizer

405 Inactivation of thermoduric sporeformers in milk by combined effect of cavitation and pasteurization. Dikshi Bawa*, Sanjeev Anand, Harsh Dahiya, and Hasmukh Patel, *South Dakota State University, Brookings, SD.*

Thermoduric sporeformers show resistance to the commonly applied thermal treatments and later produce spores during further processing of milk. They survive pasteurization and can cause spoilage defects in dairy foods. The objective of this study was to investigate the application of controlled hydrodynamic cavitation in a continuous mode, and its combination with thermal treatments on the inactivation of thermoduric sporeformers in milk. We hypothesized that the high shear created by the cavitation shock waves and the resulting friction would cause rupturing of the bacterial cell wall, resulting in cell death. Mid exponential phase vegetative cells of *Bacillus coagulans* (ATCC 12245) were inoculated in sterile skim milk at log 5 cfu/mL. Inoculated milk samples were passed at 60 Hz frequency and 200 L/h flow rate with 120 kPa back pressure, using APV Cavitator (supplied by SPX, Denmark) fitted with 4 row rotor in 6mm housing, resulting in exposure time of 22 s per pass. The inoculated milk at 10°C was recirculated until 6 passes or 6 cavitation effects (a total exposure time of 132 s) with an average inlet and outlet temperature rise up to 68°C and 82°C respectively after the 6th pass. Samples were kept in an ice bath during the treatments for temperature control. For studying the combined effect, post cavitation, samples were exposed to lab pasteurization (63°C for 30 min). Brain Heart Infusion Agar was used to plate the survivors. Experiments were conducted as replicates of 2, and were repeated thrice. Statistical significance of the data was determined using SAS software. A significant reduction ($P < 0.05$) was observed in the bacterial counts after the treatments. The 6-pass cavitation effect alone, resulted in 1.56 log survivors. Additionally, the combined effect of cavitation and pasteurization further reduced the survivors to only 0.64 log. The results revealed that 6-pass cavitation effect alone and in combination with pasteurization was very effective in inactivating thermally resistant vegetative cells of *Bacillus coagulans* by 99.963 and 99.996% respectively.

Key Words: cavitation, thermoduric sporeformers, pasteurization