

COMPETITIVE EXCLUSION BACTERIA

COMPETITIVE EXCLUSION BACTERIA AGAINST *SALMONELLA* COLONIZATION IN CHICKENS (G. Zhang, L. Ma, and M. P. Doyle)

Poultry and poultry products are important sources of human foodborne salmonellosis. Newly hatched chickens are highly susceptible to infection and colonization by *Salmonella*. Pretreatment of chicks with microflora obtained from the GI tract of adult healthy chickens can protect chicks from *Salmonella* infection; however, undefined cultures from chicken GI tract may transfer bacterial pathogens or bacteria that confer antibiotic resistance into new flocks. The objective of this project is to develop a defined competitive exclusion product that can reduce or eliminate *Salmonella* colonization in poultry.

The first step was to isolate from healthy chickens competitive exclusion bacterial candidates having antimicrobial activity to *Salmonella* in vitro. Using selective and non-selective media, 143 isolates with inhibitory activity to six *Salmonella* strains of poultry origin (2 *Salmonella* Enteritidis, 1 *Salmonella* Typhimurium, 1 *Salmonella* Heidelberg, 1 *Salmonella* Kentucky, and 1 *Salmonella* Senftenberg) were obtained from 9 donor chickens.

Seventeen trials have been carried out to test the effectiveness of many of the competitive exclusion bacterial candidates in reducing *Salmonella* colonization of chicks. The general design of these trials was similar for all, although there were slight variations between different trials. Each trial included 18 treatments, with 10 to 25 chicks per treatment. Undiluted overnight CE bacteria (approximately 10^7 - 10^8 cfu/chick) were fed to chicks at day-of-hatch and the following day. Chicks were challenged with different strains of *Salmonella* (*S.* Typhimurium, *S.* Kentucky, and *S.* Enteritidis) (ca. 10^4 cfu/chick) when they were 3 days old. They were necropsied at ca. 10 days of age. Cecal contents from each chick were enumerated on BGA plates; at the same time, they were enriched in RV broth and streaked onto BGA plates. Results were obtained for (a) the percentage of *Salmonella*-positive birds out of the total number tested in each treatment and (b) the level of *Salmonella* carriage (expressed as the geometric mean of the counts per gram of cecal content for all chicks tested in the group). CE cultures grown in MRS broth before feeding to chicks yielded inconsistent results. Modifying MRS broth to provide nutritional conditions more similar to that of the intestinal tract of chicks were *Salmonella*-positive. The effectiveness of many of the CE bacteria against *Salmonella* in chickens varied; however, six CE isolates showed consistent effectiveness against *Salmonella* colonization in chickens in repeated trials. They reduced *Salmonella* carriage in cecal content by more than $2 \log_{10}$ cfu/g on average for three trials. The percentage of chickens *Salmonella*-positive after treatment with these CE bacteria was ca. 30%, whereas ca. 80% of control (no CE bacteria treatment) were *Salmonella*-positive. Currently, we are testing different combinations of these six CE bacteria to identify the best combinations. Cultural conditions for growing the best performing CE bacteria will be optimized, and these CE bacteria will be identified and further characterized for phenotypic and genotypic properties.

SURVIVAL OF SELECTED LACTIC ACID BACTERIA IN THE BUFFER OF PACKAGED MUSSELS (C. M. Lin, Z. Yan, J. L. Kornacki, and M. P. Doyle)

Modified atmosphere packaging (MAP) is a commonly used practice in the food industry to increase shelf life. However, naturally occurring *Clostridium botulinum* in MAP foods may produce a life-threatening toxin(s) before the products are spoiled and rejected by consumers. This project was undertaken to determine the suitability of selected strains of lactic acid bacteria (LAB) for future competitive exclusion experiments to prevent *C. botulinum* in live mussels. LAB strains were screened for their ability to survive wide variations in pH, oxygen content and storage temperature in a commercial buffer solution, with and without added mussels, used to package the mussels.

Six LAB cultures were tested for growth at aerobic and anaerobic conditions on TSAYE and acidified MRS media and were also evaluated on McLung's media (a medium commonly used to recover *C. botulinum*) using an ecometric technique. All six LAB strains grew well under aerobic and anaerobic conditions.

Changes in mussel buffer pH in the presence of mussels were monitored at 3°C and 12°C, i.e., temperatures at which mussels may be stored subsequent to packaging. Mussels were shipped overnight from the manufacturer and 25 mussels (1.25 lb) were added to 500 ml of mussel buffer. The pH increased from 2.5 to 4.0 at both temperatures 1 h after combining live mussels with buffer. The pH was 4.5 at 3 h and nearly neutral at 24 h. There were no differences in pH values of mussel preparations at both temperatures at equivalent sampling times.

Changes in pH were monitored in buffer after the addition of LAB and live mussels with and without 1% glucose. Three LAB were selected and grown in MRS broth for 48 h, then 3 ml was added to 300 ml of buffer with 10 mussels. Addition of glucose did not affect the pH and the presence or absence of glucose did not influence the ability of LAB to change the pH of the buffer.

Survival of the three LAB strains in buffer with added mussels was determined. The initial cell numbers evaluated were: 1×10^9 cfu/ml for strain 43201; 1.6×10^8 cfu/ml for strain 11454; and 4.8×10^6 cfu/ml for strain 43200. LAB were enumerated on acidified MRS agar (pH 5.4). Populations of all LAB strains declined to the lowest level 2 h after they were mixed with the buffer and remained unchanged thereafter. Results revealed that populations of LAB strains were reduced in the buffer, but there was substantial survival of LAB in buffer with living mussels for 24 h. LAB strains 43201, 11454, and 43200 were reduced at 24 h by approximately 2.5, 3, and 1 \log_{10} cfu/ml, respectively.

To determine survival of a selected LAB strain in the mussel package under actual processing conditions, LAB strain 43201 was shipped to the manufacturer, and added to mussel buffer used for packaging. Strain 43201 was grown in MRS broth at 37°C for 24 h and added into the mussel buffer at ca. 1×10^6 cfu/ml. The packages were shipped to the Center for Food Safety immediately and stored at 3°C and 12°C upon receipt. The bacterial populations were monitored at 4, 7, and 11 days after packaging. Total aerobic plate counts, LAB counts, and pH values were monitored. Results revealed that LAB strain 43201 survived at ca. 1×10^5 cfu/ml throughout 11 days at both temperatures. No LAB cells were detected on MRS agar in control samples of uninoculated mussels. Total aerobic plate counts were significantly higher than controls at 7 and 11 days after packaging. There were no significant pH changes in buffer with added LAB and uninoculated control at 4 and 7 days after packaging, but at day 11 a lower pH was observed in the LAB treated packages. Temperatures did not substantially affect survival of LAB; however, total aerobic plate counts were higher at 12°C than at 3°C. Mussels in packages held at 12°C had an undesirable odor after 7 days but not at 3°C.

In summary, selected LAB strains grew well under both aerobic and anaerobic conditions. The pH of buffer with/without LAB strains increased to near neutrality within 24 h after addition of live mussels. The addition of glucose did not have a significant effect within 24 h on pH or survival of LAB strains in buffer. The maximum population decline of selected LAB strains occurred within 2 h after their addition to buffer with mussels. LAB strain 43201 survived at ca. 10^5 cfu/ml for up to 11 days after packaging in buffer with mussels when initially inoculated at 10^6 cfu/ml under actual processing conditions. Storage of mussels in buffer with LAB strains did not change the odor of mussels held at 3°C. Studies are planned to determine the ability of selected LAB strains to inhibit the growth of *C. botulinum* in mussels packaged in buffer.

This research demonstrated potential survival of selected lactic acid bacteria in a proprietary mussel packaging system. The inhibition effect of LAB strains on *C. botulinum* in mussel packages will be tested in the next phase of this work.