AGRICULTURAL RESEARCH FOUNDATION
FINAL REPORT
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TITLE: Inactivation of *Clostridium perfringens* spores adhered onto stainless steel surfaces by Clean-In-Place (CIP) procedure.

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COOPERATORS: (if any)

SUMMARY: The cross-contamination of bacteria from contaminated food-contact surfaces into finished food products is considered as one of the leading causes of food-related gastrointestinal illnesses and it can occur in food processing plant during food handling or preparation. Once attached to stainless steal (SS) surfaces, *Clostridium perfringens* cells and spores become more resistant to various commonly used disinfectants and serve as a continuous source of food contamination. Bacterial surface characteristics, such as cell surface hydrophobicity play an important role on the bacterial adhesion onto SS surfaces; thus understanding this property would lead to the development of procedure for preventing or minimizing adhesion. The Clean-In-Place (CIP) procedure had been successfully using by the food manufacturers to clean and disinfect the SS surfaces in order to control bacterial contamination. Therefore, the application of CIP procedure to inactivate *C. perfringens* cells and spores attached onto the SS surfaces is warranted.

OBJECTIVES: The objectives of this proposal were to i) Measure the surface hydrophobicity of vegetative cells and spores from various *C. perfringens* isolates, (ii) Determine the viability of *C. perfringens* spores adhered onto SS surfaces under different conditions, and (iii) Evaluate the effectiveness of the CIP procedure in removing *C. perfringens* cells and spores from SS surfaces.

PROCEDURES: A collection of *C. perfringens* FP isolates will be assessed for their hydrophobic properties. The relative surface hydrophobicity of vegetative cells and spores were measured by the BATH assay. This method based on partitioning a cell suspension between an aqueous phase and the aqueous-hydrocarbon interface relative to degree of cell surface hydrophobicity. Cell suspension (3 ml) were mixed with 0.1, 0.2, 0.6, or 1.0 ml of hexadecane and allow for phase separation 15 min before measuring the aqueous phase at absorbance 440 nm (A_{440}). The index of hydrophobicity was expressed as the average % A_{440} decrease for four hexadecane volume tested.

For measuring attachment ability, vegetative cells and spores were deposited onto SS coupons (3" x 2") and dried under sterile condition for 1 hour, placed in the sterile sealed plastic bag and stored under aerobic condition at room (20°C) and refrigerated temperature (4 ± 1 °C) which are conditions generally found in food processing plants. The number of total viable cells was determined before (1 h dried) and after 1, 3, 6, 10, 24, and 48 h storage at both temperatures by swabbing the cultures from SS surface, serially diluted, plated onto BHI agar and incubated anaerobically at 37 °C for 24 h.

The strains of *C. perfringens* FP isolates showing the maximum adherence and viability on SS coupon will be selected for evaluating the effectiveness of the CIP procedure. CIP regimes usually involve cleaning with 65 °C alkaline solution, and then disinfecting with acid treatment. Briefly, contaminated surfaces will be rinsed with sterile distilled water and then cleaned with 1% NaOH for

10 min, rinsed with sterile distilled water, disinfected with 1% nitric acid, and then finally rinsed with sterile distilled water for 5 min. All the CIP solutions will be kept at 65 °C using a water bath. The number of survival *C. perfringens* cells and spores remained on the surfaces will be determined by swabbing techniques as described above.

SIGNIFICANT ACCOMPLISHMENTS:

Survival and attachment of *C. perfringens* onto SS chips. We found that spores from both C. perfringens food poisoning (FP) and non-food-borne (NFB) isolates were survived onto SS surfaces at RT and 4°C and remained viable for up to 48 h on the attached surfaces. There was no significant differences in the survival of the spores of FP isolates at RT or 4°C, where the number of survived spores were from ~10^5 CFU/ml - ~10^6 CFU/ml. However, the survival rate for NFB spores was slightly greater than FP spores at both temperature. In contrast, vegetative cells of both FP and NFB isolates didn't survive on the SS chip at RT or 4°C, this could be attributed to oxygen sentivity and losing their resistance ability in dry conditions.

Spore and cell surface hydrophobicity. Our findings indicated that the affinity to hexadecane was dependent on the volume added to the suspension of spores or cells. Spores seemed to have higher affinity to the hexadecane than vegetative cells; spores of strain SM101 exhibited the highest hydrophobicity among 5 tested strains. The percentage hydrophobicity of spores obtained from HIC showed slightly lower surface hydrophobicity than BATH assay, the loss of spores in the eluent was due to adhering to the Sepharose gel matrix that didn't allow the spores to pass through it. Collectively, the hydrophobicity using HIC considered being similar to the hydrophobicity determined from BATH assay.

Evaluate the effectiveness of the CIP procedure in removing *C. perfringens* cells and spores from SS surfaces. This final aim of the project is currently undergoing in the lab.

BENEFITS & IMPACT: The results of this proposal provide informations on: retention and survival of *C. perfringens* spores on SS surfaces; the surface hydrophobicity of *C. perfringens* cells and spores; and the correlation hydrophobicity and adherence of this organism on SS surfaces. These findings will help validating (ongoing research) the efficacy of the commonly used CIP procedure to decontaminate *C. perfringens* cells and spores on SS surfaces.

ADDITIONAL FUNDING RECEIVED: none

FUTURE FUNDING: Explore funding from USDA.