Clostridium perfringens and safe cooling rates

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Abstracts -
Lectures & Posters

The regulatory role of HspR in Campylobacter jejuni

Poster no.: 16
Theme: Info Food

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Campylobacter jejuni is the leading cause of bacterial food-borne diarrhoeal disease throughout the world, but remains a poorly understood pathogen. Heat shock proteins fulfil important roles under both normal and environmentally stressful conditions by assisting the folding of newly synthesized or denatured proteins and in the assembly, transport and degradation of other proteins. Although heat shock proteins (Hsp) rank as some of the most conserved proteins in nature, different bacteria have evolved diverse regulatory mechanisms for controlling the expression of Hsp. Campylobacter jejuni NCTC11168 has two genes encoding homologues of the regulatory proteins HrcA and HspR that negatively regulate expression of heat shock genes in different species of bacteria. To elucidate the involvement of HspR in heat shock protein synthesis in C. jejuni, we have constructed a mutant lacking hspR and compared the proteomes of wild type and hspR mutant strains. We found increased amounts of several heat shock in the hspR mutant compared to the wild type. Surprisingly, electron micrographs of hspR mutant cells showed important morphological alterations such as loss of flagella, irregular cell division, abnormal cell-wall structure and increased size compared to wild type cells. Furthermore, the mutation reduced motility of the cells. Since motility has been implicated in pathogenesis of this organism we examined the ability of the mutant to adhere to and invade INT407 epithelial cells and found that it was greatly reduced compared to wild type cells. Thus, HspR plays diverse roles in C. jejuni including control of heat shock protein synthesis, motility, cell division and interactions with host cells.

Clostridium perfringens and safe cooling rates

Poster no.: 17
Theme: Easy Food

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Clostridium perfringens type A is one of the most commonly reported bacterial agents in foodborne disease . The vehicle of transmission is often cooked meat or poultry products in which the *C. perfringens* spores have survived the heat treatment. The heat treatment activates the spore germination system and slow cooling or hot-holding at too low temperatures enables germination, outgrowth, and multiplication. High cell levels may be reached in foods within a short period of time, and large numbers of ingested cells may sporulate in the intestine. In association with sporulation *C. perfringens* produces an enterotoxin (CPE), which is the responsible agent in food poison-

ing. Only some *C. perfringens* harbor the enterotoxin gene. The *cpe* gene can be located on either the chromosome or a plasmid, and several recent studies have revealed that all human food poisoning isolates genotyped until date carry a chromosomal *cpe* gene. The objective of the present study was to determine the growth potential after heat treatment of CPE^+ isolates carrying a chromosomal or plasmid *cpe* gene, respectively, at different constant temperatures between 20 and 50°C and at different cooling regimes. Spore germination, outgrowth and lag phase, together named GOL time, as well as generation times were determined during constant temperatures in fluid thioglycollate (FTG) medium as well as in vacuum packed, heat treated minced turkey. GOL time and growth were also followed during different cooling rates from 65°C to 10°C in minced turkey. The spores of the chromosomal CPE^+ strain were approx. 10-fold more heat resistant at 85°C and the strain had a higher temperature growth range compared with the plasmidborne CPE+ strain. A maximum acceptable cooling time of 5 h between 65°C and 10°C is suggested.

IMMUNOASSAYS FOR QUANTITATIVE MEASUREMENT OF PEDIOCIN PA-1 IN BIOLOGICAL SAMPLES

Poster no.: 18
Theme: Info Food

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Pediocin PA-1 belongs to the class II family of bacteriocins, a class of small, heat-stable, membrane-active peptides that is inhibitory for a broad spectrum of gram-positive bacteria, including spoilage and food-borne pathogens. The development of efficient immunochemical detection, quantification and purification methods for pediocin PA-1 would greatly facilitate the use of this bacteriocin as food preservative and enable its monitoring through the gastro-intestinal tract.

Polyclonal antibodies of predetermined specificity to pediocin PA-1 were generated after immunization of rabbits with two chemically synthesized peptides conjugated to carrier protein keyhole limpet hemocyanin (KLH). The chemically synthesized C-terminal peptide (22-44) and N-terminal peptide (1-9) represent the most and the less specific regions, respectively, with predicted immunogenic properties. The same peptides conjugated to thiopropyl Sepharose have been used for affinity chromatography of highly pure no-nonsense antibodies. Three pools of early low avidity, medium high avidity and late highest avidity antibodies were obtained, all with high specificity. Finally, an immunosorbent column was used as an alternative method for the purification of the natural pediocin PA-1 from culture supernatants of Lactobacillus plantarum.

The commonly used bioassay methods sometimes raise problems like lack of specificity and sensitivity. The highly sensitive sandwich ELISA method needs two pure antibodies specificities, one coated as capturing and one biotinylated, enzyme or fluorochrome conjugated as detector gave 500-1000 times more sensitive. Attempt to make bacteriocin multiplex immmunoassays are in progress.