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DNA-BASED METHODS FOR TRACING CONTAMINATION PATTERNS OF ESPECIALLY *Listeria monocytogenes*

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Listeria monocytogenes is a Gram-positive, environmental bacterium that can cause infection, listeriosis, in humans. The bacterium can cross the gastro-intestinal, the blood-brain and the placental barriers. The disease manifests itself as sepsis and meningitis and usually affects only people in specific risk groups (elderly, immuno-suppressed, the unborn feutus) and is serious with a fatality of 25-30%.

Listeriosis is a food-borne disease and typically caused by consumption of foods containing high levels of the organism. The bacterium is ubiquetous and is a common contaminant of raw materials used for food production. It is capable of multiplying at 5ºC and tolerates both NaCl and vacuum-packing. Therefore is can grow in chilled foods and cases have typically been caused by ready-to-eat (RTE) foods with extended durability.

Two intervention strategies are needed to limit or prevent listeriosis. Contamination of RTE foods must be kept at a minimum and potential bacterial growth in foods must be prevented. Determining the routes of spread of the bacterium and the contamination sites in food production has therefore become of prime importance.

We have sampled foods, processing environment and out-door environment from a range of fish processing plants. We have sampled each plant several times and both before and after cleaning and disinfection. Samples have been analysed for presence of *L. monocytogenes* and pure cultures have been isolated and identified from presumptive positive samples. Subsequently we have sub-typed the strains isolated using randomly amplified polymorphic DNA (RADP) patterns, typically using 4 different primers. Comparing sub-types of the strains have revealed that the immediate source of contamination of RTE foods typically is the processing environment itself (e.g. a slicer). We have compared and confirmed our sub-typing patterns by other molecular methods such as amplified fragment length polymorphism (AFLP), pulsed field gel electrophoresis (PFGE) and ribotyping. AFLP provides a slightly higher discrimination that the other methods but confirms the basic sub-clustering revealed by the RAPD analyses. The RAPD is a relatively simple and rapid method and does not require expensive equipment. However, great care must be taken in standardising the method with several primers to allow reproducible banding patterns.

Our longer-term samplings have shown that some food processing plants are colonized by one or a few specific sub-types than can reside in the plant for years. These sub-types do not appear to be more common in the out-door environment, and we therefore hypothesise that they carry specific genetic/phenotypic traits that allows them to persist in the food processing. We are currently investigating the physiology and genetics of these persistent subtypes.

The finding of particular contamination niches can be used by the food processing industry to develop targeted cleaning and disinfection procedures to limit or eliminate *L. monocytogenes*. This requires that the processing environment is constantly surveyed in an environmental sampling programme. Such programmes are now mandatory in several countries and do provide the basis upon which a reduction of *Listeria monocytogenes* in food processing can be obtained.