Investigation into the influence of inoculation density and temperature on the growth and enumeration of *Listeria monocytogenes*.

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Abstract

Through the use of culture based techniques, multiplex PCR and a three-strain mixture of Listeria monocytogenes *a method was developed to investigate the effect of inoculation density and temperature on growth and enumeration.*

1. Introduction

According to the Department of Agriculture, Food and the Marine (DAFM) The agri-food industry contributes €24 billion to the Irish economy and the ready to eat (RTE) fraction represents a considerable proportion of this market. However one of its single greatest challenges is the maintenance of its reputation for high quality and safety.

Listeria monocytogenes is a particular risk for the RTE food sector because it is extraordinarily well adapted to the harsh conditions employed for food preservation. Currently in foods that cannot support the growth of *L. monocytogenes* EU regulations allow up to 100 colony forming units per gram (cfu/g) of food (EU regulation 2073/2005). However, determining whether or not food can support growth can involve challenge studies combined with predictive microbiology and knowledge of the physico-chemical characteristics of the food, such as water activity and pH.

Presently the use of an inoculum density of 10^5 cfu/g of fresh weight food is common practice. However to be consistent with the EU regulations growth at 10^2 cfu/g would be needed, thus different inoculation densities from 10^2 to 10^5 were tested at two different temperatures (4°C and 8°C).

2. Methods

L. monocytogenes strain types (6179-type 1/2a, 959-type 1/2c, and 1382-type 4b/4e) were cultivated at 4°C and 8°C and colony forming units determined form optical densities at 600 nm. Specific inoculation densities (10^2 to 10^5 per g of substrate) were applied onto 10 g of Iceberg Lettuce (*Lactuca sativa*) into sealed bags with an unmodified atmosphere. On days 0, 3, 5 and 7 the lettuce was removed from the sealed bags, bacteria extracted from the food surface using a stomacher, and enumerated via Listeria Specific Agar (LSA) plates [2].

The colonies isolated on the LSA plates were then used as the source of genetic material. A PCR based assay derived from Doumith *et al* [1] was used for the serotype identification on various *L. monocytogenes* strains. A two primer multiplex assay for the confirmation of *Listeria spp*. was followed by a four primer multiplex assay for *L. monocytogenes* serotypes. This four primer assay has the ability to distinguish between the most frequently isolated serotypes 1/2a, 1/2b, 1/2c and 4b.

Approximately 50 colonies (colony count depending) were taken from each LSA plate on the respective days (Day 0 and Day 7) allowing for a comprehensive identification of the strain composition on the plates.

3. Results

Growth of the three strain mix of *L. monocytogenes* at 8°C over the 7 day period showed a maximum increase of 1.53 log units. At 4°C over the 7 day log period showed a maximum increase of 1.95 log units. Growth rate was unaffected by the inoculation density, as difference for each density applied a minimum of 1 log unit of growth was recorded. Preliminary results suggest that strain type 6179 has the highest rate of detection (41.58%) from the multiplex PCR assay, with strain 1382 (34.65%) and strain 959 (23.76%) completing the strain mix. However, there was no significant correlation between temperature and inoculation density on the detection of the various strain types at this time.

4. Conclusion

From the preliminary information gathered from this set of experiments it can be established that for *L. monocytogenes* strains 969, 6179, and 1382 that inoculation density between 10^2 and 10^5 has little affect on the growth at temperatures of 4°C and 8°C, thus past inoculation experiments using higher inoculation densities did not introduce any major experimental biases.

5. References

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