Proposition de stage

Parcours Master 2 « Microbiologie, Environnement, Santé »

1. **Laboratoire / Entreprise d'accueil :**

Intitulé : Anses, Laboratoire de sécurité des aliments de Maisons Alfort
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Perspectives de poursuite de thèse :

o oui
x non

avec une bourse spécifique
o oui
x non

2. **Titre, description du sujet, approches utilisées, références** (2 pages maximum) **:**

Title: Development of a method to study growth limits of Listeria monocytogenes at low temperature

Introduction:

With regard to consumer safety, the benefit of cold chain is to limit growth of pathogens that need to reach high levels in food to cause illness. Among these, the most relevant pathogens are those able to grow at cold temperature (i.e. the most difficult to control by the cold chain). This mainly corresponds to *Listeria monocytogenes*. It is a robust, ubiquitously present foodborne human pathogen and the causative agent of listeriosis. This pathogen has served as a model in a large number of studies that addressed the impact of strain diversity (3) and the role of population heterogeneity in adaptive stress response and survival capacity (1).

An increased number of studies in quantitative microbiology have shown that lag time and probability of growth is much more uncertain and difficult to predict compared to growth rate (2, 4). However, the validity of a mathematical model in predicting the conditions that lead to critical levels in foods highly depends on its ability to describe the effect of the environment

on lag time and growth probability. This implies that a better understanding of the pathogen behavior (lag time and growth probability) near growth limits is of great importance for the effective application of predictive models in food safety risk assessment.

The classical method for individual cell lag times and growth probability acquisition is based on turbidity measurement on microplate wells inoculated with approximately one bacterial cell per well (5). The growth probability can be determined either by monitoring visually 96-microwell plates and deduced from concentrations estimated with the MPN calculation (4) or by comparing the ratio of cfu formed on agar plates incubated in studied conditions to cfu obtained in optimal conditions (2). Yet these methods are labour intensive, long and would benefit of a higher throughput.

In order to improve throughput of data collection at single cell level, a direct method should be developed. It will consist in the use of direct cell observation with a phase-contrast microscopy (equipped with a 100× objective and a high-resolution device camera) (6). This device will be used to study growth initiation and lag phase of individual cells deposited on microscopic slides covered by agar. Automation of image acquisition on large surface will be used to reach a high throughput.

With this method the single cell growth probability will be characterized at low temperature. Experimental effort will be concentrated on 0-4°C range. The number of strains and conditions (different temperature profiles), that will be studied will be chosen according to the analytical capabilities provided.

Finally the obtained data will be analyzed statistically and distributions will be fitted.

1. Abee, T., J. Koomen, K. Metselaar, M. Zwietering, and H. d. Besten. 2016. Impact of Pathogen Population Heterogeneity and Stress-Resistant Variants on Food Safety. Annual Review of Food Science and Technology.
2. Aguirre, J. S., and K. P. Koutsoumanis. 2016. Towards lag phase of microbial populations at growth-limiting conditions: The role of the variability in the growth limits of individual cells. International Journal of Food Microbiology 224:1-6.
3. Aryani, D. C., H. M. W. den Besten, W. C. Hazeleger, and M. H. Zwietering. 2015. Quantifying strain variability in modeling growth of Listeria monocytogenes. International Journal of Food Microbiology 208:19-29.
4. Augustin, J.-C., and A. Czarnecka-Kwasiborski. 2012. Single-cell growth probability of Listeria monocytogenes at suboptimal temperature, pH and water activity. Frontiers in Microbiology 3:157.
5. Guillier, L., and J.-C. Augustin. 2006. Modelling the individual cell lag time distributions of Listeria monocytogenes as a function of the physiological state and the growth conditions. International Journal of Food Microbiology 111:241-251
6. Koutsoumanis, K. P., and A. Lianou. 2013. Stochasticity in colonial growth dynamics of individual bacterial cells. Applied and Environmental Microbiology 79:2294-2301.