London Metropolitan University

Aspects of modelling variability of single cell lag time for *Cronobacter* spp. after exposure to sublethal heat treatment in normal and stressful environments

This Thesis is submitted in partial fulfilment of the requirements for the degree of Doctor of Philosophy

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DELARATION

I confirm that this is my own work and the use of all material from other sources has been clearly acknowledged.

(Signature)

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ABSTRACT

The growth profiles of five strains of *Cronobacter* spp. at low temperature (4 ^{o}C to 8 ^{o}C) and their thermal resistance to mild heat treatment (48 ^{o}C to 50 ^{o}C) were investigated with a view to developing a model to predict lag times of individual cells, using a strain with a relatively high thermotolerance and able to grow well at refrigeration temperature (7 ^{o}C).

The effect of heat stress (49 ^{o}C for 7 min) and subsequent recovery temperatures (7 ^{o}C to 22 ^{o}C) on the individual cellular lag times of one strain of *Cronobacter turicensis* were analysed using optical density measurements. It was found that the distribution of individual lag times of *Cr. turicensis* shifted right and became more spread when the recovery temperature decreased, and the distribution was more skewed after heat stress. Assuming the lag of a single cell follows a shifted Gamma distribution with a fixed shape parameter, the effect of recovery temperature on the individual lag times of untreated and sublethally heat stressed cells of *Cr. turicensis* were modelled. It was found that the *shift* parameter *(T_{shift})* increased asymptotically as the temperature decreased, while the logarithm of the scale parameter *(θ)* decreased linearly as recovery temperature increased.

To test the validity of the model, growth of low numbers of untreated and heat stressed *Cr. turicensis* in tryptone soy broth (TSB) and infant first milk was measured experimentally between 7 ^{o}C and 22 ^{o}C and compared with predictions obtained by Monte Carlo simulations. It was found that in TSB, in most cases, the simulations from both models underestimated the actual growth of individual cells of *Cr. turicensis* from challenge tests; while in first milk, the untreated model slightly underestimated the actual growth at low temperatures (7 ^{o}C and 12 ^{o}C). The heat stressed model in first milk was generally in agreement with the data derived from the challenge tests and provides a basis for reliable quantitative microbiological risk assessments for *Cronobacter* spp. in infant milk.

The study has made a contribution to understanding and modelling the responses of untreated and sublethally heat stressed *Cr. turicensis* at 7 ^{o}C, 12 ^{o}C and 22 ^{o}C at single cell level.

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LIST OF ABBREVIATIONS

$\alpha(1)$ = physiological state of single cell

$\alpha(N_0)$ = physiological state of the initial population consisting of N_0 cells

ρ = parameter of Poisson distribution; the average cell number per well in Bioscreen

$\rho+$= parameter of zero truncated Poisson distribution; the average cell number per positive well in Bioscreen

τ = parameter for Lag (1); it follow Gamma distribution with shape parameter β and scale parameter θ

β = shape parameter of Gamma distribution

θ = scale parameter of Gamma distribution

a_w= water activity

ANOVA= Analysis of variance

BA = blood agar plates

BACANOVA = Bacterial lag time using analysis of variance techniques

BLAST =Basic Local Alignment Search Tool

BHI = brain heart infusion

cfu=colony forming unit

C. = Citrobacter

Cr. = Cronobacter

CV = coefficient of variation

DFI = Druggan-Forsythe-Iversen agar

DNA= deoxyribonucleic acid

DNase= deoxyribonuclease

dNTP= deoxyribonucleotide triphosphate

dOD= the difference between two consecutive OD readings

$\mathrm{d_t}$ = the time interval between two OD readings.

D-value = decimal reduction time

EDTA = ethylene diamine tetraacetic acid

EE = Enterobacteriaceae enrichment

Ent. = Enterobacter

Esch. = Escherichia

EVIIb = extreme value type II distribution, with a shape parameter fixed to 5

f-AFLP = fluorescent-amplified fragment length polymorphism

FAO = Food and agriculture organization of the United Nations

FCA = fecal coliform agar

FDA= Food and Drug Administration

Fig = figure

Fluorocult ECD = Fluorocult *E. coli* direct agar

rDNA=ribosomal deoxyribonucleic acid

rRNA = ribosomal RNA

h=hour

h_0 = work to be done

HACCP = Hazard Analysis and Critical Control Point

HIA = Heart Infusion Agar

H_20_2 = hydrogen peroxide

HPA = Health Protection Agency

L. = *Listeria*

Lag(1) = the single cell lag time

Lag(N_0) = population lag produced by N_0 cells

LBDC = Leuschner, Baird, Donald, and Cox agar

Ln = Natural logarithm

LSA= *Listeria* selective agar

M = mol/l

Max = Maximum value

$MgCl_2$=Magnesium chloride

min = minute

Mini = minimum value

ml = millilitre

MLST = multilocus sequence typing

mmol= millimolar

MPN= most probable number

MRD = maximum recovery diluent

MRU = Microbiology Research Unit

MRSA = methicillin-resistant *Staphylococcus aureus*

MSc = Master of Science

N= cell concentraton

NA = nutrient agar

NaCl = sodium chloride

NCTC = National Collection of Type Cultures

NCIMB= National Collection of Industrial Food and Marine Bacteria

N_{det} = cell number at detection time

$N(t_c)$ = cell number at time t_c

ng = nanogram

N_0= initial cell number

N_{max}= maximum cell density

NICU = neonatal intensive care units

OD = optical density

OK= Oh and Kang agar

PCR = polymerase chain reaction

PIF = Powdered infant formula

PMP = Pathogen Modelling Program

R^2 = coefficient of determination of regression

RMS= root mean square

rpm = revolutions per minute

S. enterica = *Salmonella enterica*

S_α = the sum of the physiological states of the individual cells in a well.

SD = Standard Deviation

SE= Standard Error

Staph. aureus = *Staphylococcus aureus*

T = temperature variable

T_{det} = detection time

T_{shift} = shift parameter for *Lag(1)*

TBE = Tris-Borate-EDTA

TGE = tryptone-glucose extract agar

TSA = tryptone soya agar

TSAP= tryptone soya agar supplemented with sodium pyruvate

TSAPA = tryptone soya agar supplemented with sodium pyruvate before autoclaving

TSAPF = tryptone soya agar supplemented with sodium pyruvate after autoclaving

TSB =tryptone soy broth

ppm = parts per million

U=unit

UK=United Kingdom

USA= United States of America

USDA= US Department of Agriculture

µm=micrometre

µg = microgram

µl = microlitre

µm=micromolar

µ' = specific growth rate

VRBGA = violet red bile glucose agar

W_0 = the number of negative wells in the Bioscreen

W = the total well number in the Bioscreen

WHO = World Health Organization

XαGlc = 5-bromo-4-chloro-3-indolyl-α, D-glucopyranoside

χ^2 test = the Chi-square test

z value = increase in temperature needed for one log reduction in the D-value.