UNIVERSITI PUTRA MALAYSIA

EFFECTS OF SUBLETHAL TEMPERATURE STRESS ON THE GROWTH, SURVIVAL AND CULTURABILITY OF LISTERIA MONOCYTOGENES

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MOHD NIZAM BIN LANI

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfillment of the Requirement for the Degree of Master of Science

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To:

my ayahanda and bonda

my brothers; Daus, Oshin, Afiz, Md Nor and Adik Im

my sisters; Angah, Khairiah and Azma

my relatives, lecturers and friends

for their love and support who have been inspiring my life

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirement for the degree of Master of Science

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Chairman : Abdul Reezal bin Abdul Latif, Ph.D.

Faculty : Food Science and Biotechnology

Survival and growth of *Listeria monocytogenes* L56 (IMR isolate) was studied in trypticase soy broth grown at 37°C before being subjected to three selected sublethal stress of temperatures (55°C, 28°C and 4°C) using log and stationary phase as inoculums using two-plating systems; TSA with and without 4% NaCl (TSAS). The influence of morphological changes and listerial motility as affected by sublethal stress of temperatures were also determined using Scanning Electron Microscopy (SEM) and motility media, respectively. A standard growth curve of *L. monocytogenes* at 37°C was established using plate counts showed that the log and stationary phase of the organism were achieved after 12 and 19 hours, respectively. It was observed that viable bacterial population (CFU/ml) after log and stationary phase were 10^8 and 10^9, respectively. From the growth curve, the generation time of *L. monocytogenes* at 37°C was 60 min.

The bacterial growth rates obtained from culturability on culture plates assessed using two-system media, TSA with and without 4% NaCl concentration (TSAS) were assessed by their generation time. Cells of *L. monocytogenes* grown in exponential phase cultures demonstrated biphasic survival curves at 55°C and 4°C in both media. In contrast, survival curves at 28°C were not biphasic. The growth rates of *L. monocytogenes* grown in stationary phase cultures were also assessed by their generation time. The addition of sodium chloride enhanced heat resistance of microorganism.

It has been proven that biphasic curve and tailing with/without shoulder from thermal inactivation curves in this study were associated with the occurrence of microbial injury. During the exponential phase of *L. monocytogenes*, the percentage injury at 55°C, 28°C and 4°C were ranged between 3.21% to 28.49%, 2.47% to 4.38%, and 4.34% to 8.61%, respectively. Whilst, during the stationary phase of *L. monocytogenes*, the percentage injury at 55°C, 28°C and 4°C were ranged between 2.05% to 4.15%, 1.44% to 3.06%, and 1.07 to 4.25%, respectively.

L. monocytogenes cells were able to survive throughout the sublethal stress of temperatures and undergone morphological changes to adapt to new temperatures. In this study, results from Scanning Electron Microscopy (SEM) revealed three different analyses of temperature-stressed cells which were average mean of cells length, distribution of cells length, and minimum versus maximum cells length. The study demonstrated cells of both log and stationary phase showed a significant variation of morphology. Cells of log phase became elongated only at 55°C, not at 28°C and 4°C whereas cells of stationary phase were shorter and more coccoidal rather than elongated

as in log phase cells. However, cells at 28°C were more intact than cells at other temperatures for most of the observations.

In conjunction with SEM results, the variation of listerial morphology and the effect of listerial motility would be a part of microbial adaptation towards sublethal stress of temperatures. The loss of motility in stationary phase cells strongly suggested that listerial motility play a role in survival of the organism under temperature stress. The stationary phase cells of *L. monocytogenes* were more resistant than exponential phase cells exhibited by increased of generation times, lower percentage injury and most of the cells became coccoid.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia
sebagai memenuhi keperluan untuk ijazah Master Sains

KESAN TEKANAN SUHU 'SUBLETHAL' TERHADAP PERTUMBUHAN, KEMANDIRIAN DAN KEUPAYAAN KEKULTURAN *LISTERIA MONOCYTOGENES*

Oleh

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November 2002

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Kemandirian dan pertumbuhan *Listeria monocytogenes* L56 (sumber organisma daripada IMR) telah dijalankan di dalam trypticase soy broth yang telah dihidupkan pada suhu 37°C sebelum dikenakan tekanan suhu 'sublethal' terhadap tiga suhu yang terpilih (55°C, 28°C and 4°C) daripada fasa eksponensial dan fasa statik sebagai inoculum berbeza menggunakan dua sistem piring agar yang berbeza; TSA dengan dan tanpa 4% NaCl (TSAS). Selain itu, kesan pengaruh morfologi dan pergerakan *Listeria* disebabkan oleh tekanan suhu juga dikaji menggunakan Mikroskopi Pengimejan Elektron dan media pergerakan. Graf pertumbuhan yang seragam pada suhu 37°C telah dilakukan menggunakan pengiraan koloni piring petri, di mana didapati fasa eksponensial dan fasa statik pada suhu 37°C telah diperolehi selepas 12 dan 19 jam dengan pertumbuhan jumlah populasi bakteria dianggarkan sebanyak 10^8 and 10^9

(CFU/ml), masing-masing. Daripada graf pertumbuhan bakteria, masa generasi *L. monocytogenes* pada suhu 37°C ialah 60 minit.

Kadar pertumbuhan bakteria yang diperolehi daripada kekulturan di atas piring petri menggunakan dua sistem media berbeza, iaitu media yang mengandungi dan tanpa mengandungi kepekatan garam sebanyak 4 peratus (TSA and TSAS) dinilai berasaskan masa generasi. Sel *L. monocytogenes* yang dihidupkan dalam fasa eksponential menunjukkan graf yang mempunyai dua keluk pada suhu 55°C dan 4°C dalam kedua-dua media. Sebaliknya, graf pertumbuhan kesan suhu pada suhu 28°C tidak mempunyai dua keluk. Walaubagaimanapun, kadar pertumbuhan *L. monocytogenes* yang dihidupkan pada fasa statik juga dinilai berdasarkan masa generasi. Penambahan garam telah meningkatkan kerintangan haba mikroorganisma tersebut.

Ia telah dibuktikan bahawa kehadiran dua keluk dan kesan 'tailing' beserta dan tanpa 'shoulder' terhadap rintangan haba dalam kajian ini telah mempunyai kaitan dengan kehadiran mikroorganisma yang tercedera. Semasa pertumbuhan fasa eksponensial *L. monocytogenes*, peratus kecederaan pada suhu 55°C, 28°C dan 4°C di dalam julat 3.21% hingga 28.49%, 2.47% hingga 4.38%, dan 4.34 hingga 8.61%, masing-masing. Manakala, semasa pertumbuhan fasa statik bagi *L. monocytogenes*, peratus kecederaan pada suhu 55°C, 28°C dan 4°C ialah pada julat 2.05% hingga 4.15%, 1.44% hingga 3.06%, dan 1.07 hingga 4.25%, masing-masing.

Sel-sel *L. monocytogenes* telah menunjukkan keupayaan merintangi tekanan suhu 'sublethal' dan mengalami perubahan morfologi semasa penyesuaian suhu baru. Dalam

kajian ini, keputusan daripada Mikroskopi Pengimejan Elektron (MPE) telah menunjukkan tiga analisis berbeza terhadap sel-sel tekanan suhu seperti purata kepanjangan sel, taburan kepanjangan sel, serta kepanjangan minimum dan maksimum sel. Kajian ini menunjukkan kedua-dua fasa eksponensial dan fasa statik menunjukkan variasi morfologi yang signifikan. Sel-sel daripada fasa eksponensial telah menjadi panjang hanya pada suhu 55°C, tidak pada suhu 28°C dan 4°C, manakala sel-sel pada fasa statik adalah pendek dan lebih banyak berbentuk 'coccoid' berbanding sel yang panjang pada fasa eksponensial. Walaubagaimanapun, morfologi sel-sel pada suhu 28°C tidak terjejas berbanding dengan suhu-suhu lain pada kebanyakan pemerhatiannya.

Bersama-sama dengan keputusan MPE, variasi terhadap morfologi *Listeria* dan kesan pergerakan *Listeria* merupakan sebahagian daripada penyesuaian mikrobiologi yang dilakukan terhadap tekanan suhu 'sublethal'. Kehilangan keupayaan pergerakan semasa fasa statik dengan tegasnya mencadangkan pergerakan *Listeria* memainkan peranan penting dalam kemandirian organisma terhadap tekanan suhu. Didapati, fasa statik *L. monocytogenes* adalah lebih resistan berbanding sel yang berada pada fasa eksponensial berdasarkan peningkatan nilai masa generasi, peratus kecederaan yang rendah dan kebanyakan sel telah menjadi 'coccoid'.

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LIST OF ABBREVIATIONS

CAMP Test	(Christie, Atkins, and Munch-Peterson) Test
CDC	Centres for Disease Control and Prevention
CFU	Colony forming unit
D value	Decimal Reduction Time
FDA	Food and Drug Administration
NaCl	Sodium Chloride
RCPA	Royal College of Pathologists of Australasia
SEM	Scanning Electron Microscopy
TSA	Trypticase Soy Agar
TSAS	Trypticase Soy Agar with added 4% Sodium Chloride
TSB	Trypticase Soy Broth
z value	Reciprocal Slope Value of Thermal Death Time Curve

CHAPTER I

INTRODUCTION

Listeria monocytogenes was presumably first isolated at the beginning of the twentieth century as a Gram positive rod in tissue specimens of infected patients (Fsihi *et al.*, 2001). In 1919, Hulphers called *Bacillus hepatis* the bacterium he isolated from necrotic foci in rabbit liver. In 1926, *L. monocytogenes* was described by Murray and colleagues as *Bacterium monocytogenes* in connection with an epizootic among laboratory-raised guinea pigs and rabbits (Murray *et al.*, 1926). However, the following year, Pirie described a similar epizootic in wild gerbils termed "Tiger River Disease" and proposed that the species to be called *Listerella hepatolytica.* After finding that *Bacterium monocytogenes* and *Listerella hepatolytica* was the same organism, the name was changed to *Listeria monocytogenes.* The current name *Listeria* was suggested in 1940 by Pirie in honour of Lord Lister, an eminent English surgeon and discoverer of antiseptics (Seeliger and Jones, 1986).

L. monocytogenes is appreciably more heat resistant than other non-sporing microorganisms such as *Salmonella* and *Campylobacter*, and can grow at refrigeration temperatures (Farber and Peterkin, 1991). The bacterium is ubiquitous in nature and can be isolated from a wide variety of sources, both the environment and food. It also appears to be a common environmental contaminant of food processing plants. The foods that have received most attention have been milk and dairy products, meat, poultry, seafood and vegetables as *L. monocytogenes* is capable of survival and growth