



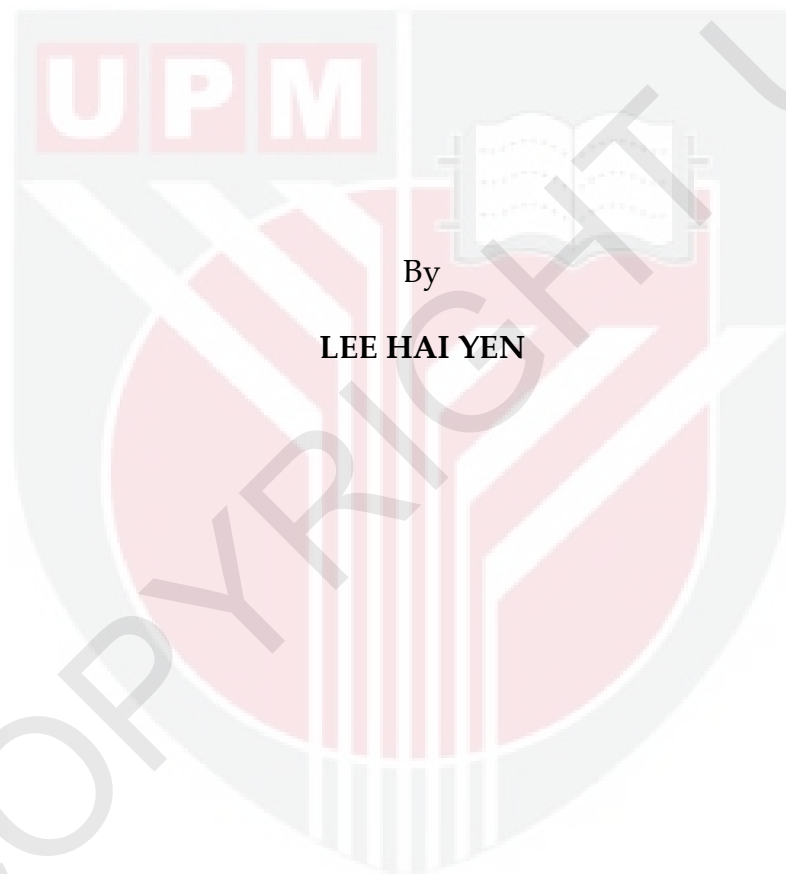
**UNIVERSITI PUTRA MALAYSIA**

***EXPOSURE ASSESSMENT OF *Listeria monocytogenes* AND BIOFILM  
FORMATION IN READY-TO-EAT CHEESE***

**LEE HAI YEN**

**FBSB 2011 50**

**EXPOSURE ASSESSMENT OF *Listeria monocytogenes* AND BIOFILM  
FORMATION IN READY-TO-EAT CHEESE**



By

**LEE HAI YEN**

**Thesis submitted to the School of Graduate Studies, Universiti Putra  
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**December 2011**

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirement for the degree of Doctor of Philosophy

**EXPOSURE ASSESSMENT OF *Listeria monocytogenes* AND BIOFILM FORMATION IN READY-TO-EAT CHEESE**

By

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**December 2011**

**Chairperson: Shuhaimi Mustafa, PhD**

**Faculty: Faculty of Biotechnology and Biomolecular Sciences**

Cheese has been implicated in many different outbreaks of human listeriosis worldwide through consumption of contaminated cheese that occurs at farm and processing levels. The risk involved in consumption of a contaminated cheese can be evaluated through exposure and risk assessment. A total of 214 samples of cheeses were obtained from various point of entry (importing ports) in Malaysia as well as cheese from retail stores in five different states were assessed for prevalence and enumeration of *L. monocytogenes*. The concentration of *L. monocytogenes* in the samples were applied to model the exposure assessment on the contaminated food samples obtained from the entry point to the processing factory, and from the factory to the consumer's home prior consumption. Different scenarios were included in the exposure

assessment model to show the effect of temperature, pH and water activity in the growth of *L. monocytogenes*. In previous studies, persistence and contamination of food products in the food processing environment may be attributed to the biofilm formation by *L. monocytogenes*. It was found that biofilm formation occurs at high level of sodium chloride, different temperatures over a period of 60 h. At 4°C, biofilm formation occurs more rapidly. The cross contamination of *L. monocytogenes* can occur from formation of biofilm on food grade stainless steel to plastic wrappers through contact. The transfer of *L. monocytogenes* from stainless steel to polypropene plastic wrappers showed that biofilm and colonies of *L. monocytogenes* can be transferred through contact surface, regardless of weight applied on the surface type. This showed a steady contamination rate in the processing factory when biofilm is found on the surface. The removal of biofilm and colonies of *L. monocytogenes* using laboratory designed cleaning-in-place (CIP) system using water, commercial detergent and bleach, showed that removal of colonies was effective using the commercial detergent and bleach ( $P>0.05$ ) and has a log reduction of 1 to 1.5 log cfu/ml. The design of CIP did not remove the biofilm on the surface of stainless steel using water and detergent, but bleach concentration of 10% (5.55% active compound of sodium hypochlorite) were able to significantly reduce the density of biofilm

on the stainless steel surface. The preliminary risk assessment showed that worst case scenario of listeriosis are highest in immunocompromised individuals and can be significantly reduced through intervention strategies such as the CIP cleaning for removal of biofilm from the stainless steel surface and reducing the cross contamination of food products.



Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

**PENILAIAN PENDEDAHAN OLEH *Listeria monocytogenes* DAN  
FORMASI BIOFILEM DALAM KEJU SEDIA DIMAKAN**

Oleh

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Keju telah diimplikasikan di dalam beberapa wabak listeriosis manusia di seluruh dunia melalui pemakanan keju tercemar yang berlaku di peringkat ladang dan pemprosesan. Risiko yang terlibat dalam pemakanan keju tercemar boleh dinilai melalui penilaian pendedahan dan risiko. Sejumlah 214 sample keju telah diperolehi dari beberapa titik kemasukan (port impot) di Malaysia dan juga di kedai di lima negeri berlainan telah diuji untuk kelaziman dan pengiraan *L. monocytogenes*. Jumlah *L. monocytogenes* dalam sampel telah diaplikasikan untuk model penilaian pendedahan terhadap

sampel makanan yang tercemar yang diperolehi daripada titik kemasukan kepada kilang pemprosesan, dan dari kilang pemprosesan kepada rumah pengguna sebelum dimakan. Beberapa senario telah dimasukkan kedalam model penilaian pendedahan untuk menunjukkan kesan suhu, pH dan aktiviti air dalam pertumbuhan *L. monocytogenes*. Dalam kajian lepas, persisten dan kontaminasi bahan makanan dalam persekitaran pemprosesan makanan boleh disebabkan oleh pembentukan biofilem oleh *L. monocytogenes*. Ia telah ditemui bahawa pembentukan biofilem berlaku pada tahap kandungan sodium klorida yang tinggi, pada suhu yang berbeza dalam jangka masa 60 jam. Pada suhu 4°C, pembentukan biofilem berlaku dengan lebih cepat. Kontaminasi bersilang *L. monocytogenes* boleh berlaku dari pembentukan biofilem pada keluli tanpa karat gred makanan kepada pembalut plastic melalui sentuhan. Pemindahan *L. monocytogenes* dari keluli tanpa karat kepada pembalut plastik polypropene menunjukkan bahawa biofilem dan koloni-koloni *L. monocytogenes* boleh dipindahkan melalui sentuhan permukaan, tidak mengira berat yang diletakkan pada jenis permukaan. Ini menunjukkan kadar kontaminasi yang mantap di kilang pemprosesan apabila biofilem ditemui pada permukaan. Penyingkiran biofilem dan koloni *L. monocytogenes* menggunakan 'cleaning-in-place' (CIP) yang direka di makmal menggunakan air, detergen komersial dan peluntur,

menunjukkan penyingkiran koloni adalah efektif dengan menggunakan detergen dan peluntur ( $P>0.05$ ) dan mempunyai log pengurangan 1 sehingga 1.5 log cfu/ml. Rekabentuk CIP tidak dapat menyingkirkan biofilem pada permukaan keluli tanpa karat dengan menggunakan air dan detergen, tetapi, dengan peluntur kepekatan 10% (5/55% bahan aktif sodium hipoklorait) dapat mengurangkan dengan banyak kepadatan biofilem pada permukaan keluli tanpa karat. Penilaian risiko awal menunjukkan kes yang paling teruk untuk kes listeriosis adalah paling tinggi bagi pesakit yang kekurangan daya tahan badan dan boleh dikurangkan dengan banyak melalui strategi campur tangan seperti pembersihan CIP untuk penyingkiran biofilem dari permukaan keluli tanpa karat dan mengurangkan kontaminasi bersilang dalam produk makanan.



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I would also like to take this opportunity to dedicate the thesis to my family, who probably would never expected me to go this far in life (ha ha ha, proved you wrong again!), especially to my father, although you left us a bit too early, but we all have ended up very well. For my mother and sister who sacrificed everything for the rest of us; to put clothes on our back and meal on the table, knowledge into our head, attitude and perseverance in life, thank you for not 'getting' in my way even though I was selfish and stubborn. I hope my achievements will make all of you proud of me today, for what I was made me who I am.

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Thank you to everyone.



## Approval sheet 1

I certify that an Examination Committee has met on \_\_\_\_\_ to conduct the final examination of Lee Hai Yen on her Doctor of Philosophy thesis entitled “Exposure assessment of *Listeria monocytogenes* in ready-to-eat cheeses” in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the candidate be awarded the relevant degree. Members of the Examination Committee are as follows:

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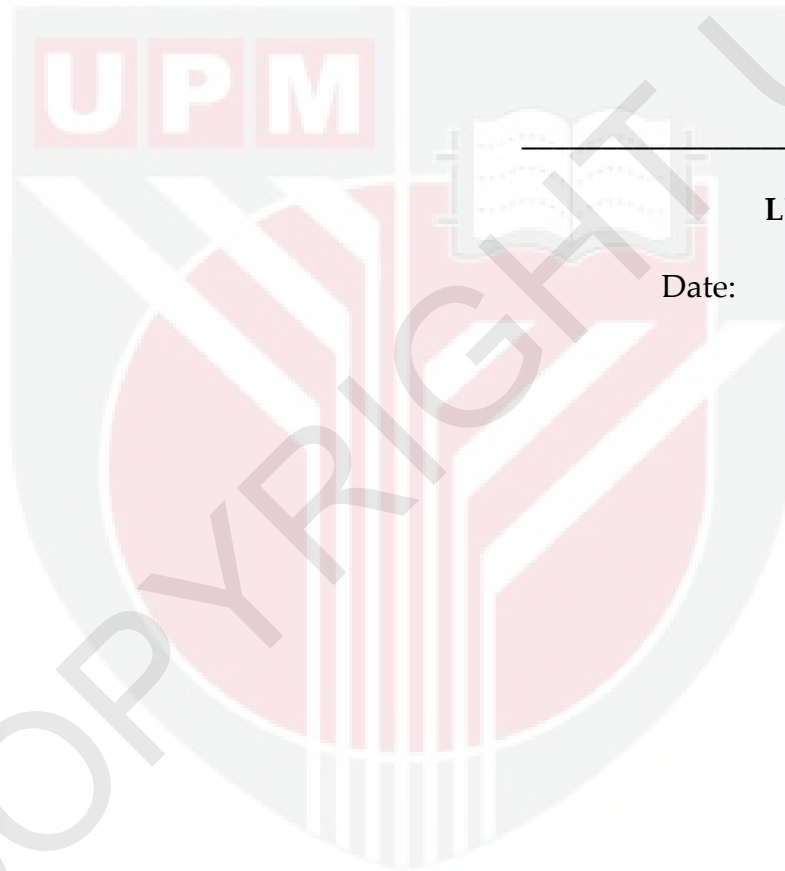
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## DECLARATION FORM

I declare that the thesis is my original work except for quotations and citations which have been duly acknowledged. I also declare that it is not been previously, and is not concurrently, submitted for any other degree at Universiti Putra Malaysia or other institutions.



\_\_\_\_\_  
**LEE HAI YEN**

Date:

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