UNIVERSITI PUTRA MALAYSIA

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By

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CHARACTERIZATION OF *Bacillus cereus* ISOLATED FROM READY-TO-EAT CEREALS

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APRIL 2009

Chairman : Farinazleen Mohamad Ghazali, PhD

Faculty : Faculty of Food Science and Technology

Since availability of data on the contamination of spore formers such as *B. cereus* in ready-to-eat foods is scarce particularly from developing countries, this surveillance was conducted to address the issue. The preliminary findings from this study revealed a high prevalence of *B. cereus s.l.* being detected using MPN-PCR in 76% of 111 samples of ready-to-eat cereals tested. The range of concentration was from 30 MPN/g to more than 24,000 MPN/g. Results indicated the differences in the level of contamination for *B. cereus s.l.* in various products based on factors such as product types, ingredients added and location of manufacturer. The highest concentration of *B. cereus s.l.* was found in samples with ingredients from weaning products which are, product made from vegetable origin.

The alarming findings on the high prevalence of *B. cereus s.l.* in ready-to-eat cereals prompted further studies on the isolates from these samples. Isolation of colonies from these samples were characterized based on toxin gene screening, plasmid profiles, antibiotic resistance and fingerprinted using RAPD-PCR analysis. In addition to the high prevalence in RTE cereals, the toxin screening profile indicated 58% of the isolate carry the *Bacillus* enterotoxin T (*bceT* gene) and 34% carries the tri-component non-hemolytic enterotoxin (*nhe* gene). This shows that majority of the isolates from ready-to-eat cereals are diarrheagenic. The plasmid profile revealed one isolate carrying the plasmid size similar to cereulide protein which is responsible for the emetic disease therefore showing the less common prevalence of emetic isolates from RTE cereals. Isolates of *B. cereus s.l.* were found to be resistant to ampicillin, metronidazole but highly susceptible to antibiotics with mechanism of action that inhibits the protein synthesis such as erythromycin, oxytetracycline, spectinomycin, neomycin, furozolidone, quinopristin/dalfopristin. Even though *B. cereus* usually manifests a self limiting disease, the emergence of antibiotic resistance bacteria is a major concern worldwide and due to ease of horizontal gene transfer between the

subspecies of *B. cereus s.l.,* the risk of other more lethal subspecies such as *B. anthracis* obtaining the antibiotic resistance gene may also occur.

This preliminary finding revealed an interesting risk profile for *B. cereus s.l.* as there are no known data available for the assessment on the microbiological quality of RTE cereals especially for spore formers. Based on the risk assessment study conducted, an estimate of on worst case scenario showed 48 diarrhea cases in 27 million populations are known to occur per annum from consumption of RTE cereals.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk Ijazah Sarjana

PENCIRIAN *Bacillus cereus* YANG DIPENCILKAN DARI BIJIRIN SEDIA DIMAKAN

Oleh

Pengerusi : Farinazleen Mohd. Ghazali, PhD

Fakulti : Fakulti Sains dan Teknologi Makanan

ABSTRAK

Sejak kesediaan data mengenai kontaminasi pembentuk spora seperti *B. cereus* dalam bijirin sedia dimakan adalah terhad terutamanya dari negara yang sedang membangun, pengawasan ini telah dijalankan untuk mengalamatkan isu tersebut. Hasil penyelidikan preliminari daripada kajian ini menunjukkan kekerapan *B. cereus s.l.* yang dikesan menggunakan MPN-PCR dalam 76% daripada 111 sample bijirin sedia dimakan yang telah diuji. Jajaran konsentrasi didapati adalah dari 30 MPN/g sehingga melebihi 24,000 MPN/g. Keputusan ini menunjukkan perbezaan dalam tahap kontaminasi bagi *B. cereus s.l.* dalam pelbagai produk berdasarkan faktor-faktor seperti jenis produk, ingredients tambahan serta lokasi manufacturer. Konsentrasi *B. cereus s.l.* yang tertinggi didapati dalam sampel yang mengandungi produk 'weaning' iaitu produk yang dihasilkan dari sayuran.

Keputusan yang menggelisahkan mengenai kekerapan *B. cereus s.l.* yang tinggi daripada bijirin sedia dimakan telah menyaran ujikaji lanjutan ke atas pencilan koloni dari sampel-sampel makanan. Pemencilan koloni-koloni diciri berdasarkan pemeriksaan gen toxin, profail plasmid, kerintangan antibiotic serta analisis RAPD-PCR fingerprint. Profail yang didapati dari kajian ini menunjukkan 58% daripada pencilan mengandungi *Bacillus* enteroxin T gen (*bceT* gen) dan 34% daripada pencilan mengandungi tiga komponen 'non-hemolytic enterotoxin (*nhe* gen). Ini menunjukkan bahawa majoriti daripada pencilan dari bijirin sedia dimakan adalah jenis cirit. Profail plasmid memperlihatkan satu pencilan yang mengandungi plasmid saiz yang serupa kepada protein 'cereulide' yang menyebabkan muntah-muntah oleh itu menunjukkan kekerapan yang kurang lazim untuk pencilan jenis muntah dari bijirin sedia dimakan. Pencilan *B. cereus s.l.* ditemui menjadi rintangan kepada ampisilin dan metronidazole tetapi mudah dipengaruhi oleh antibiotik dengan mekanisma tindakan yang mencegah sintesis protein seperti erythromycin, oxytetracycline, spectinomycin, neomycin, furozolidone dan quinopristin/dalfopristin. Walaupun *B. cereus* menyebabkan penyakit batas sendiri, kemunculan bakteria rintangan antibiotic adalah satu perhatian utama di dunia. Oleh kerana kemudahan pemindahan horisontal gen antara subspesis *B. cereus s.l.*, risiko subspesis

yang lebih maut seperti *B. anthracis* memperolehi gene kerintangan antibiotik mungkin juga akan berlaku.

Hasil penemuan preliminari menunjukkan satu profil risiko yang menarik bagi *B. cereus s.l.* kerana ketiadaan data untuk penilaian kualiti mikrobiologi untuk bijirin sedia dimakan terutamanya bagi pembentuk spora. Berdasarkan kajian penilaian risiko yang dijalankan, taksiran bagi senario paling buruk menunjukkan 48 kes cirit dalam 27 juta populasi berlaku setiap tahun daripada pemakanan bijirin sedia dimakan.

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I have to thank God for everything, for being able to come this far; I thank God for giving me strength and courage when I falter; thank God for my family because they taught me to be me and believe in myself even if I have to defy them; thank God for the fate that brought me to my supervisor (Dr. Farin) and co-supervisor (Prof Son), which opened up this road and bring me a step closer to my dreams; thank God for my friends (especially Lay Ching who is always there for everything, Grace for keeping my off lab hours sane, Siow Yin and Wai Yee for their support) because they have exposed me to many lab and life experiences that helped me grow; thank God for putting me through difficult and embarrassing moments to keep me humble and braver; and thank God for keeping me alive so I will always know how little I have and how precious things are and keep moving forward with these cherished ones.

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and knowledge shared will always be cherished and remembered for the rest of my life and beyond.

This thesis is especially dedicated to my three elder sisters and mother for sacrificing everything because even at their most difficult trying times, they are still able to love the most difficult person on earth. Thank you so much for still loving me especially through the times when I can't even love myself and for those difficult times, you have made me what I am today.

Thank you once again, for there is no known depth for my gratitude.

I certify that an Examination Committee has met on 14th April 2009 to conduct the final examination of LEE HAI YEN on her Master of Science thesis entitled "Characterization of *Bacillus cereus* isolated from ready-to-eat cereals" in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Act 1981. The committee recommends that the student be awarded the Master of Science in Food Safety.

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfillment of the requirement for the degree of Master of Science in Food Safety. The members of the Supervisory Committee were as follows:

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Date: 8 June 2009

DECLARATION

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

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LEE HAI YEN

Date: 24 July 2009

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LIST OF ABBREVIATIONS

BAM	Bacteriological Analytical Manual
bceT	Bacillus cereus enterotoxin T
DNA	Deoxyribonucleic acid
GMP	good manufacturing practice
HACCP	hazard analysis critical control point
MPN	Most Probable Number
MYP	Mannitol Yolk Polymyxin
NHE	non-hemolytic enterotoxin
PCR	Polymerase chain reaciton
RAPD	Randomly Amplified Polymorphic DNA
RBC	red blood cell
rpm	revolution per minute
RTE	ready-to-eat
s.l.	*sensu lato*
s.s.	*sensu stricto*
VP	Voges-Proskauer
WHO	World Health Organization

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CHAPTER 1

INTRODUCTION

The resilience of spore formers has become a major concern in food safety. Particularly for *Bacillus cereus sensu lato* which is the parent group of six subspecies (*Bacillus cereus sensu stricto, Bacillus anthracis, Bacillus thuringiensis, Bacillus mycoides, Bacillus pseudomycoides* and *Bacillus weihenstephanensis*), this group has increasingly getting attention because what initially thought as the *B. cereus s.s* as the only foodborne pathogen, it has been found that other subspecies such as *B. thuringiensis* may have accounted to the outbreaks caused by *B. cereus s.s* (Jackson et al., 2008). This is due to the fact that the subspecies does not only possess a high homology in genomic DNA sequences, the prevalence of toxins and different symptoms it manifests also differs within the single subspecies such as *B. cereus s.s.* For example, three various strains of *B. cereus s.s.* have been analyzed due to the involvement in the recent several outbreaks and fatalities (Lund et al., 2000) and another type of strain for food poisoning that does not produce any known toxin (Lapidus et al., 2008). Recently, anthrax-like toxin possessed by *B. cereus* isolates has been detected to cause severe respiratory illnesses (Hoffmaster et al., 2004) from food. Toxins from other subspecies such as *B.*

weihenstephanensis, a psychrotrophic strain surviving causing problems in milk contamination were also found to be involved other foodborne outbreaks (Stenfors Anrnesen et al., 2008).

Since the changing trend of pathogenesis on *B. cereus* extends to its six subspecies, this issue calls for a greater concern and re-assessment on products not only as ready-to-eats but also the fact that these foods are also consumed by the more vulnerable individuals such as children and convalescing patients. Therefore, it is important to have at least a baseline data to serve as a guide for developing a system on food safety monitoring so that food hazards can be identified in an early stage to prevent the unwanted outcomes. These data requires information on characteristics and behavior as well as point of entry into the food chain (Kleter and Marvin, 2008).

In comparison to the other types of pathogens such as *Salmonella* spp., *Campylobacter* spp., *E. coli* O157:H7 and *L. monocytogenes,* data availability of *B. cereus s.l.* as part of surveillance study in developing countries are scarce or not available. To date, there is only one appraisal of microbiological quality of ready-to-eat cereals by Rani et al. (2005) but that did not include spore formers which have attributed to several outbreaks of severe foodborne

poisoning as well as fatal outbreaks found in infant foods. Despite the sporadic outbreaks of *B. cereus* in foods, there are no known surveillance data available particularly from the developing region.

The practice of microbial risk assessment is increasingly applied in the assessment of food hazards because of the changes in foodborne diseases such as lower infectious dose of 10^3 to 10^4 cells *B. cereus* have been reported to be responsible for an outbreak case (Notermans et al., 1997). MRA data on *B. cereus* is scarce in the recent years indicating the need to address and update the evolving hazards of *B. cereus* in foods. In Malaysia, the Ministry of Health has recently established the Food Safety and Quality division in 2002 to monitor the food safety issues in Malaysia. The first microbiological risk assessment report was published on the contamination of *Vibrio parahaemolyticus* in tiger prawns, which aided the country's management of food quality when facing international trade regulations. This shows the importance of risk assessment studies which generates data not only for the national surveillance but also for the international bodies.