UNIVERSITI PUTRA MALAYSIA

DETECTION AND QUANTIFICATION OF BACILLUS CEREUS AND BACILLUS THURINGIENSIS FROM COOKED RICE AND BURGER EMPLOYING MOST PROBABLE NUMBER-POLYMERASE CHAIN REACTION (MPN-PCR) METHOD

AFRIANI SANDRA

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By

AFRIANI SANDRA

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

May 2012

Dedicated for my father, late mother, my husband, my daughter and all my beloved family for the endless love and support

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

DETECTION AND QUANTIFICATION OF *BACILLUS CEREUS* AND *BACILLUS THURINGIENSIS* FROM COOKED RICE AND BURGER

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May 2012

Chairman : Professor Dr. Son Radu, PhD

Faculty : Food Science and Technology

The aim of this study was to determine the prevalence and number of *Bacillus cereus* and *Bacillus thuringiensis* in cooked rice and burgers in Serdang, Selangor, Malaysia. A combination of Most Probable Number - Polymerase Chain Reaction (MPN-PCR) method was applied to detect the presence of total *B. cereus* and *B. thuringiensis* harboring *gyrB* genes. The biosafety of *B. cereus* were assessed by antibiotic resistance test of the isolates from both cooked rice and burger samples. A kitchen simulation study was conducted to investigate germination of *B. cereus* with the storage condition similar to domestic kitchens (ambient temperature). Estimation of the risk of illness and risk rating from consumption of cooked rice and burger were

investigated using semi-quantitative Risk Ranger spreadsheet tool. The prevalence of *B. cereus* group was relatively high in cooked rice with ranged of 50% to 100% and burger 26.6% to 46.2%. The prevalence of *B. thuringiensis* was 10% to 35.2% for cooked rice and 10.8% to 33.9% for burger. However, none of raw rice samples were positive for *B. thuringiensis* but all samples were positive for *B.cereus*. The study was extended to investigate the level of contamination in cooked rice. A total comprised of 115 samples of *nasi lemak* (n=54), *nasi briyani* (n=20), *nasi ayam* (n=20) and *nasi putih* (n=21) were collected from mention the places. *Nasi ayam* was found to have higher prevalence (100%) of *B. cereus* compared to *nasi putih* (76.2%) and *nasi lemak* (70.4%), while *nasi briyani* have lowest prevalence (50%). Only 10% of *nasi putih* and *nasi ayam*, 30% of *nasi briyani* and 35.2% of *nasi lemak* samples were found to harbor *B. thuringiensis*. Furthermore, 197 burger samples were analyzed. Fish burgers had higher contamination level (46.2%) compared to beef burgers (41.5%) and chicken burgers (26.6%) for *B. cereus.* Prevalence of fish burgers had lowest contamination level for *B. thuringiensis* (10.8%) compared to chicken burgers (13.9%) or beef burgers (33.9%). A total of 92 *B. cereus* isolates were recovered by plating method and confirmed by PCR. Antibiotic resistance profiling indicated that multi-resistance to *B. cereus* might be wide-spread in the study area. The isolates showed multi-resistance to 8 antibiotics tested, with 98.9% resistance to ampicillin and mostly

susceptible to norfloxacin (1.1% resistance). High Multiple Antibiotic Resistance (MAR) indices were detected in this study (ranging from 0 to 0.63) with more than 54.3% of the isolates had a MAR index value of 0.36 and 42.4% showed MAR index of 0.25. At domestic kitchen level, simulation on the germination of *B. cereus* by storing cooked rice was carried out. In this study the simulation was designed to imitate real events in domestic kitchens as much as possible to give a realistic quantitative data on how *B. cereus* spores can germinate while storing at ambient temperature. The four storing times (0, 6, 12, and 18 h) were applied in the simulation study. Tap water was used to wash raw rice before cooking procedures and dishwater from washing rice was investigated to test if washing step can reduce contamination of *B. cereus* in raw rice. In this study, both naturally and artificially contaminated raw rice were used. The mean concentration of *B. cereus* in the samples after storage for 18 h was 2.8 MPN/g both for washing and without washing raw rice. Number of *B. cereus* ranged from 460 MPN/g up to 1100 MPN/g values. It was found that rinsing can greatly reduce the number of *B. cereus* in raw rice samples (up to 0.88 log reduction). The potential of cooked rice and burgers as a *B. cereus* transmitter was demonstrated by semi-quantitative Risk Ranger spreadsheet. Based on the assumptions used in the risk ranger, *nasi putih* had the highest predicted cause of illness. It was assumed that *nasi putih* is a staple food in Malaysia

with average consumption of 2x/day. The risk ranking is 47 with predicted illnesses of 1800 per annum. Considering Malaysian population of 28.7 million, it is assumed that 75% of population consumes *nasi putih*. However, the risk estimate was predicted to be reduced when less people consuming the contaminated foods. In conclusion, the results suggested that cooked rice and burger act as a transmission route for *B. cereus* and thus pose a risk for consumers. Further studies on a bigger scale are recommended for a better understanding on the presence of *B. cereus* in cooked rice and burgers and the risks involved in consumption of such foods.

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

PENGESANAN DAN PENGKUANTITIAN *BACILLUS CEREUS* DAN *BACILLUS THURINGIENSIS* DARIPADA NASI DAN BURGER

Oleh

AFRIANI SANDRA

Mai 2012

Chairman : Profesor Dr. Son Radu, PhD

Faculty : Sains dan Teknologi Makanan

Matlamat kajian ini adalah untuk menentukan prevalens dan kuantiti daripada kumpulan *Bacillus cereus* (*Bacillus cereus* (*B. cereus)* and *Bacillus thuringiensis (B. thuringiensis)*) dalam nasi dan beberapa jenis burger di kawasan terpilih di Selangor, Malaysia. Suatu kombinasi kaedah Jumlah Paling Mungkin – Reaksi Polimer Berantai (MPN-PCR) telah diterapkan untuk mengesan kehadiran *B. cereus* dan *B. thuringiensis* yang mempunyai gen *gyrB*. Bio keselamatan *Bacillus cereus* telah dinilai oleh ujian rintangan antibiotik pencilan - pencilan dari kedua-dua sampel. Satu kajian simulasi dalam dapur telah pun dijalankan untuk mengkaji pertumbuhan *B. cereus* yang disimpan di dapur domestik (pada suhu ambient). Anggaran risiko

penarafan penyakit dan risiko pemakanan nasi dan burger disiasat menggunakan "semi-kuantitatif spreadsheet Risk Ranger". Prevalens kumpulan *B. cereus* dalam nasi dan burger amat tinggi dengan masing-masing 50% - 100% untuk nasi dan 26.6% - 46.2% untuk burger. Prevalen *B. thuringiensis* adalah 10% - 35.2% untuk nasi dan 10.8% - 33.9% untuk burger. Walau bagaimanapun, tiada sampel beras yang positif untuk *B. thuringiensis* tetapi semua sampel didapati positif *B.cereus*. Kajian ini telah diperluaskan untuk menyiasat tahap pencemaran di dalam nasi. Sebanyak 115 sampel nasi yang terdiri dari pada nasi lemak (n=54), nasi briyani (n=20), nasi ayam (n=20) dan nasi putih (n=21) telah pun dikumpulkan. Nasi ayam didapati mempunyai prevalens (100%) yang lebih tinggi terhadap *B. cereus* berbanding dengan nasi putih (76.2%) atau nasi lemak (70.4%), sementara nasi briyani mempunyai prevalens yang lebih rendah (50%). Hanya 10% sampel nasi putih dan nasi ayam, 30% nasi briyani dan 35.2% nasi lemak telah dikontaminasi oleh *B. thuringiensis*. Sementara itu, 197 sampel burger telah pun dianalisis dan keputusan kajian mendapat burger ikan mempunyai tahap pencemaran *B. cereus* yang lebih tinggi (46.2%) berbanding burger daging (41.5%) atau burger ayam (26.6%). Burger ikan mempunyai aras kontaminasi yang terendah bagi *B. thuringiensis* (10.8%) berbanding dengan burger ayam (13.9%) atau burger daging (33.9%).

Sebanyak 92 pencilan *B. cereus* diperoleh menggunakan kaedah piring dan disahkan oleh PCR. Profil kerintangan antibiotik menunjukkan bahawa kerintangan berganda *B. cereus* mungkin tersebar dengan meluas di kawasan kajian. Pencilan-pencilan tersebut menunjukkan kerintangan berganda terhadap 8 antibiotik yang dikaji, dengan kerintangan 98.9% terhadap ampicillin dan kebanyakannya terdedah kepada Norfloxacin (1.1% kerintangan). Indeks Kerintangan Antibiotik Berganda (MAR) yang tinggi telah dikesan dalam kajian ini (berkisar daripada 0 sehingga 0,63), dengan lebih 54.3% pencilan mempunyai indeks MAR 0,36 dan 42.4% menunjukkan indeks MAR 0.25. Pada kajian ini, simulasi telah direka untuk meniru sebanyak mungkin peristiwa sebenar di dapur domestik untuk memberikan data kuantitatif yang realistik bagaimana spora *B. cereus* boleh bercambah dengan menyimpannya dalam suhu ambien. Empat waktu penyimpanan (0, 6, 12 dan 18 jam) telah pun di jalankan. Air paip digunakan untuk membasuh beras sebelum kaedah memasak dan air basuhan beras disiasat untuk menguji langkah membasuh dapat mengurangkan pencemaran *B. cereus* dalam beras. Dalam kajian beras yang dikontaminasi secara semula jadi dan buatan digunakan. Purata kepekatan *B. cereus* dalam sampel selepas disimpan selama 18 jam adalah 2,8 MPN/g dengan membasuh ataupun tanpa membasuh beras. Jumlah *B. cereus*

berkisar diantara 460 MPN/g sehingga 1100 MPN/g. Ianya didapati bahawa membasuh beras sangat mengurangkan jumlah *B. cereus* pada sampel beras (log pengurangan sehingga 0,88). Potensi nasi dan burger sebagai pembawa *B. cereus* dalam penyebaran penyakit telah ditunjukkan oleh "semi-kuantitatif, spreadsheet Risk Ranger". Berdasarkan andaian-andaian yang digunakan dalam *Risk Ranger,* nasi putih mempunyai punca yang tertinggi diramalkan menyebabkan penyakit. Oleh itu dianggap nasi putih ialah makanan yang umum di Malaysia. Kedudukan risiko adalah 47 dengan kes yang diramalkan 1800 setahun. Kaedah ini mengambil kira penduduk Malaysia sebanyak 28.7 juta dan diandaikan bahawa 75% daripada penduduk makan nasi putih. Walau bagaimanapun, ramalan anggaran risiko dapat dikurangkan apabila pengambilan nasi yang terjangkiti diambil berkurang. Sebagai kesimpulan, hasil kajian mencadangkan bahawa nasi dan burger bertindak sebagai bahan transmisi untuk *B. cereus* dan dengan itu menimbulkan risiko kepada pengguna. Kajian selanjutnya pada skala yang lebih besar disyorkan untuk pemahaman yang lebih baik mengenai kehadiran *B. cereus* dalam nasi dan burger, dan risiko yang terlibat sekiranya mengambil makanan tersebut.

ACKNOWLEDGEMENTS

Alhamdulillahhirabbil'alamiin. First of all I would like to express my utmost thanks and gratitude to the Almighty Allah S.W.T, for providing me the blessings and the easiness to complete this work. My Salawat and Salam is addressed to his righteous messenger, prophet Muhammad S.A.W.

I submit my highest appreciation to the chairman of my supervisory committee, Professor Dr. Son Radu who helped in every step of the way. Thank you very much for support and guidance throughout my study, and for always believing in me and seeing the potential more than the weaknesses.

A million thanks to Dr. Farinazleen Mohamad Ghazali, my co-supervisor. My deepest appreciation goes to you for the encouragement, advice and support that you gave endlessly. And I would like to special thanks to Dr. Leili Afsah Hejri for advice and edited of my thesis. Thank you so much for your guidance and patience.

Sincere thanks to Tunung Robin and Tuan Zainazor, who is also a senior in the Food Safety and Quality Lab, for her assistance in any way that I may have asked. Thank you for the time and energy that you have spent to help me and to mentor my work. You have been very good friend to me.

I would also like to say thank you to all of my dearest friends and lab mates Indah, Chui Mei, Margaret, Dr. John, Zarrul, Kak Noorlis, Chai Fung Dr. Chai Lay Ching, Dr. Natasha, Hidayah and others for their kindness and moral support during my study. Thanks for the friendship and memories.

I am also indebted to my Indonesian colleagues (Indonesian Students Association members). Special thanks, tribute and appreciation to all those their names do not appear here who have contributed to the successful completion of my study.

Finally, a thousand heartfelt thanks to my late mother. Although you are not with us anymore, I know that you are still watching us from the heaven. I can still feel your love for us. Also, to my father, without your support I will never have moved this far in my life. Thank you for your endless love and support for me. I love you so much! Special thanks to my husband, for supporting me morally, for listening to my problems, and for everything. Also for my daughter, Assyifa, you a good girl. I love you all! However, the writer feels many shortcomings in this report, so the writer expects the suggestion, advice and critic from the readers.

I certify that an Examination Committee has met on 29 Mei 2012 to conduct the final examination of Afriani Sandra on her Master of Science thesis entitled "Detection And Quantification Of *Bacillus cereus* And *Bacillus thuringiensis* From Cooked Rice And Burger By Using Most Probable Number-Polymerase Chain Reaction (MPN-PCR)" in accordance with Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the degree of Master of Science.

Members of the Thesis Examination Committee were as follows:

Tan Chin Ping, PhD
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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Master of Science. The members of the Supervisory Committee were as follows:

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Date:

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 nor concurrently, submitted for any other degree at Universiti Putra Malaysia or any other institution.

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AFRIANI SANDRA

Date: 29 May 2012

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