UNIVERSITI PUTRA MALAYSIA

MOLECULAR CHARACTERIZATION OF LISTERIA SPP. ISOLATED FROM BEEF, CHICKEN AND FERMENTED FISH IN MALAYSIA

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FSMB 2003 36

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2003

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By

HAJJAH ENDANG PURWATI RAHAYUNINGSIH

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirement for the Degree of Doctor of Philosophy

January 2003

DEDICATIONS

To my late mother, Rr. Soejatningsih ,

My late father, R. Soejono and my mother in law Hajjah Mursiati for their help

and prayers .....

To my husband, papa Dr. Mursof Fauzi Saladin MD, O & G

and my daughter Sofrida Prigustina Kurniasari

for their love, understanding, prayers and patience .....

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

MOLECULAR CHARACTERIZATION OF *LISTERIA* SPP. ISOLATED FROM BEEF, CHICKEN AND FERMENTED FISH IN MALAYSIA

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January 2003

Chairman: Dr. Hajjah Zaiton Hassan

Faculty : Food Science and Biotechnology

Modified FDA method was found to give higher recovery of *Listeria* spp. than USDA method for different sources. The results indicated that the imported frozen beef samples from wet market examined were contaminated by seven different *Listeria* spp. namely, *L. monocytogenes, L. ivanovii, L. seeligeri, L. innocua, L. welshimeri, L. grayi* and *L. murrayi.* However, the use of FDA, USDA and the modified USDA methods may be more beneficial where a limited range of *Listeria* spp. to be recovered (*L. monocytogenes, L. ivanovii* and *L. innocua*). *Listeria* spp. was not isolated from any of the 23 samples of imported frozen beef from supermarket and from the five samples of buffalo meat examined.

A total of two hundred and seventy isolates of *Listeria* spp. from different sources were investigated for their susceptibility to 18 antibiotics and were screened for plasmid profiles. Antibiotic susceptibility analysis revealed that all *Listeria* spp. isolates were resistant to two or more antibiotic (MAR 0.11 to 0.66). Majority of the 52 isolates of *L. monocytogenes* displayed resistance to bacitracin, ceptazidime, nalidixic acid, sulfamethazole. However, none were resistant to norfloxacin. Plasmid was detected in 9 (81.8%) of 11 strains of *L. murrayi*, 12 (80%) of 15 strains of *L. grayi*, 29 (55.8%) of 52 strains of *L. monocytogenes*, 8(50%) of 16 strains of *L. denitrificans*, 25 (41.7%) of 60 strains of *L. ivanovii*, 25 (37.9%) of 66 strains of *L. innocua*), 13 (31%) of 42 strains of *L. welshimeri* and 1(12.5%) of 8 strains of *L. seeligeri.* The plasmid sizes ranged from 2.7 to 54 Kb.

In the conjugation study, the donor and the recipient were selected based on the antibiotic resistance pattern of selected *Listeria* spp. isolates from different sources. Seven *L. monocytogenes* strains and one *L. innocua* strain isolated from different sources were selected as donors since they were expected to potentially have an ability to conjugate due to the presence of high molecular weight plasmid DNA (54 Kb). Plasmidless sensitive to antibiotics are selected as recipients. Streptomycin resistance was transferred to *L. monocytogenes* LM65 and LM100 strains at frequencies of

3.3×10^{-8} and 1.2×10^{-9} per input donor cells. A selected kanamycin resistant *L. innocua* strains was found to transfer kanamycin resistant to *L. monocytogenes* (inter-and intra-species transfer).

The use of randomly amplified polymorphic DNA (RAPD) analysis for characterization and differentiation of the isolates of different *Listeria* spp. was examined. The combination of the RAPD-PCR patterns obtained with the three primers (Gen15001, Gen15002 and Gen15010) were able to distinguish all typeable isolates. Base on the dendrograms generated from the RAPD-PCR patterns all *Listeria* spp. isolates could be discriminated and clustered according to their species and food sources. RAPD fingerprinting methods had higher resolution than plasmid profiling and antibiotic resistance patterns and therefore the RAPD fingerprinting methods can be used for epidemiological studies.

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

PENCIRIAN SECARA MOLEKULAR KEATAS *LISTERIA* SPESIS YANG DIPENCILKAN DARIPADA DAGING LEMBU, DAGING AYAM DAN IKAN YANG DITAPAI DI MALAYSIA

Oleh

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Kaedah pengubahsuaian FDA telah didapati memberikan pulangan yang tinggi untuk spesis *Listeria* berbanding kaedah USDA daripada sumber yang berbeza. Keputusan menunjukkan bahawa sampel daging lembu beku import yang diuji telah dicemarkan oleh tujuh spesis *Listeria* iaitu *L. monocytogenes, L. ivanovii, L seeligeri, L. innocua, L. welshimeri, L. grayi* dan *L. murrayi.* Walau bagaimanapun, kegunaan kaedah FDA, USDA dan USDA diubahsuai mungkin lebih menguntungkan dimana spesis *Listeria* yang terhad boleh didapati (*L. monocytogenes, L. ivanovii* and *L. innocua*). Spesis *Listeria* tidak dapat dipencilkan daripada mana-mana 23 sampel daging lembu beku import dari supermarket dan 5 sampel daging kerbau yang dikaji.

Sejumlah dua ratus dan tujuh puluh isolat spesis *Listeria* daripada sumber yang telah diperiksa untuk kerektanan kepada 18 jenis antibiotik dan diskrin untuk mendapatkan profail DNA (asid deosiribonuklik) plasmid. Analisis kerektanan antibiotik menunjukkan bahawa kesemua isolat spesis *Listeria* adalah rentang kepada 2 atau lebih antibiotik (MAR 0.11 hingga 0.66). Kebanyakkan 52 isolat *L. monocytogenes* mempamerkan kerintangan kepada basitrasin, septazidim, nalidixik asid, sulfamethazole. Walau bagaimanapun, tiada kerintangan kepada norfloxasin. Plasmid telah dikesan di dalam 9 (81.8%) dari 11 strain *L murrayi*, 12 (80%) dari 15 strain *L. grayi*, 29 (55.8%) dari 52 strain *L. monocytogenes*, 8 (50%) dari 16 strain *L. denitrificans*, 25 (41.7%) dari 60 strain *L. ivanovii*, 25 (37.9%) dari 66 strain *L. innocua*, 13 (31%) dari 42 strain *L. welshimeri*, 1 (12.5%) dari 8 strain *L. seeligeri*, telah menunjukkan kehadiran DNA plasmid. Saiz plasmid berukuran dari 2.7 hingga 54 kb.

Dalam kajian konjugasi, penderma dan penerima telah dipilih berdasarkan corak kerentangan antibiotik spesis *Listeria* yang dipencilkan daripada sumber yang berbeza. Tujuh strain *L. monocytogenes* dan satu *L. innocua* yang dipencilkan daripada sumber berbeza telah dipilih sebagai penderma memandangkan mereka mempunyai potensi dan keupayaan

konjugasi disebabkan kehadiran berat molekul DNA plasmid yang tinggi (54kb) dan penerima mestilah tiada plasmid dan sensitif kepada antibiotik yang dipilih.

Penggunaan analisis DNA polimorfik penggandaan secara rawak (RAPD) untuk pencirian dan pembezaan isolat daripada spesis *Listeria* yang dikaji. Kombinasi corak fingerprinting RAPD yang diperolehi daripada 3 primer (Gen15001, Gen15002 and Gen15010) telah digunakan untuk pembezaan semua isolate. Daripada dendrogam yang digenerasi semua spesis *Listeria* boleh dibezakan dan dikumpulan bergantung kepada spesis dan sumber makanan mereka. Kaedah fingerprinting RAPD adalah lebih sensitif berbanding kepada corak kerintangan antibiotik. Oleh kerana itu, ia digunakan untuk kajian epidemiologi.

ACKNOWLEDGEMENTS

My grateful thanks is due to Dr. Zaiton Hassan, the Chairman of the Supervisory Committee, for her patience, encouragement, persuasion and guidance in the preparation of this thesis and her helpful counseling during the course of this study.

A word of thanks to Associate Professor Dr. Son Radu and Associate Professor Dr. Raha Abdul Rahim, members of the Supervisory Committee, for their facilities, invaluable guidance and advice throughout the thesis preparation. Thanks also goes to Professor Dr. Gulam Rusul Rahmat Ali for discussion and for research materials.

I also wish to acknowledged SEAMEO-SEARCA for providing the thesis grant award.

I wish to express my appreciation to Andalas University, in particular the Rector and Dean of Faculty of Animal Husbandry, for approving my study leave to complete my project.

I am indebted to Dr. Lum Keng Yeang, Dr. Sahilah Abdul Mutalib and Kak Rosma from MARDI for help, facilities and advice on Gel Compar for RAPD analysis.

My sincere thanks is also due to Associate Professor Dr. Ahmad Ismail and Encik Ruslin from Kolej 13 Universiti Putra Malaysia and Bapak Yazid from Embassy Indonesia in Kuala Lumpur for their help and facilities during my study.

I wish to thank Mr. Ali Alkatiri, Ir. Raja Ruslan Raja Sharif, Dr. H. Jamal Ali Johari MD, Dr. Imam Rahayu and their families for support and prayers.

Thanks are also due to my fellow graduate students, especially Maimunah, Zainuri, Samuel, Ooi Wai Ling, Kqueen , Haryanti, Noorlis, Rozila and many others, for help and involvement in discussion during my study.

Above all, my praise to Almighty God, Allah SWT. for all His blessing on me and my family.

This thesis submitted to the Senate of Universiti Putra Malaysia has been accepted as fulfilment of the requirements for the degree of Doctor of Philosophy. The members of the Supervisory Committee are as follows:

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DECLARATION

I hereby declare that the thesis is based on my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at UPM or other institutions.

_____

Hajjah Endang Purwati Rahayuningsih

Date:

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