# ANALYSIS OF DNA IN *PASTEURELLA HAEMOLYTICA* A2,A7,A9 AND T

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TERENGGANU

1999/2000

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2000

**JABATAN BIOLOGI**
**FAKULTI SAINS DAN TEKNOLOGI**
**UNIVERSITI PUTRA MALAYSIA TERENGGANU**

**BORANG PENGESAHAN DAN KELULUSAN**
**LAPORAN TAHUN AKHIR**

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PROGRAM : **Bacelor Sains (Kepujian) Biologi** NO. MATRIK : **UK 950**

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TAJUK PROJEK : **Analysis Of Dna In *Pasteurella Haemolytica* A2, A7, A9 And T**

Dengan ini disahkan bahawa saya telah menyemak laporan projek ini dan

i. semua pembetulan yang disarankan oleh pemeriksa-pemeriksa telah dibuat

ii. laporan ini telah mengikut format yang diberikan dalam Panduan BIO 4999 (Projek) Jabatan Sains Biologi, Fakulti Sains dan Teknologi, Universiti Putra Malaysia Terengganu.

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**(Dr. Mohd. Effendy Abd. Wahid)**
Penyelia Utama

Tarikh : 16 April 2000

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Penyelia Kedua

Tarikh : _____

# ANALYSIS OF DNA IN *PASTEURELLA HAEMOLYTICA* A2, A7, A9 AND T

**BY :**

**SITI RUHAYA BINTI ABDUL MANAF**

**This project report is submitted in partial fulfillment of the requirements for the degree of Bachelor of Science (Honours) in Biology**

**DEPARTMENT OF BIOLOGICAL SCIENCES**
**FACULTY OF SCIENCE AND TECHNOLOGY**
**UNIVERSITI PUTRA MALAYSIA**
**TERENGGANU**

**2000**

*SPECIAL DEDICATED TO :*

*Abah (Abdul Manaf Bin Hidayat)*
*&*
*Emak (Aminah Binti Kayat)*

*Abang (Amin, Iwan, Li, Daud & Kamal)*
*Kakak (Ani & Ana)*
*Adik (Siti)*
*Abang ipar & Kakak ipar*

*Abdul wahab Hj. Abdullah*

*" Ct Aya "*

*15 MARCH 2000*

## ACKNOWLEDGEMENT

**In the name of AllAH, the most Precious, the most Merciful**

Alhamdulillah, My deepest thanks to Allah The Almighty, for HIS guidence and will, I was able to finish my final year project on B. Sc (hons) Biology.

My utmost appreciates to my respected supervisor, Dr. Mohd. Effendy Bin Abdul Wahid and co-supervisor, Prof. Madya Dr. Mohd. Zamri Bin Saad, for their valuable knowledge, guidence, advises, encourage and patience throughout to completing my project. And a greatest grateful to Dr. Anum Man, Dr. Md. Sabri Mohd Yusof, Dr Yuslan Sanuddin, Encik Mohd. Jamil Abd. Samad, Tuan Haji Mohd. Noh Manap, Dr. Kamaruddin Mat Isa and Encik Ismail Mohd. Shairi (Histology lab, Faculty of veterinary UPM, Serdang) and all lecturers of biology in UPMT, thanks a lot for all your kindness, teaching and help.

Also a big thanks to my entire friend, who always been there with me in my goodness and sadness times, especially to all my coursemates and roomates. Special thanks to Kak Emma, Joned, Kelvin, Tina, Ai kim and Rajeni, we were sharing our happily and sadly times together for a years to cheerful our project and finally with our hardworks and patience, we were able to complete our greats final year project, WELL DONE. May our memories that we were spent together will remain in our heart forever.

To my beloved family, especially Abah, Emak, Abang, kakak, adik, all kakak ipar and abang ipar, I would like to express my gratitude a million thanks for their encouragement, supported, moral and materials for me throughout to achieve what I have got now. And for someone special ... my fiance, Abdul Wahab Hj. Abdullah, Thanks a lot for being my mentor and inspirations. Thank you for your true love.

And Last but not least, thanks to all related individuals that helped me directly or indirectly, to finish my project. Hope ALLAH s.w.t could repaid your kindness.

# ABSTRAK

Tujuan kajian ini dijalankan adalah untuk menilai sejauhmana keberkesanan kegunaan teknik Analisis Pembatas Endonukleas (REA) dalam proses pengecaman dan pengenalpastian serotip *Pasteurella haemolytica* biotip A dan T.

Empat replikasi pengasingan *Pasteurella haemolytica* serotip A2, A7, A9 dan biotip T telah dipencilkan dari peparu bebiri yang dijangkiti pasteurellosis pneumonia. DNA bagi setiap pengasingan bakteria kemudiannya diekstrak dengan menggunakan sistem "Wizard® genomic purification kit". Analisis REA dijalankan sebanyak 4 kali dengan menggunakan enzim pembatas *Hae* III. Gelung DNA dibandingkan dengan menggunakan 6 penanda major yang terpilih (marker LambdaDNA/*Hind* III). Saiz berat molekul kemudiannya dikira dengan memplotkan graf garis lengkung linear.

Dari keputusan proses REA yang dijalankan menggunakan serotip yang homologus menunjukkan pemisahan yang sama dalam setiap 4 replikasi pengasingan pada setiap serotip *Pasteurella haemolytica* A2, A7, A9 dan T. Walaubagaimanapun terdapat gelung DNA yang khusus dan lazim yang ditemui hadir pada semua kumpulan serotip iaitu pada gelung 16.8 kbp, 13.7 kbp, 10.7 kbp, 8.5 kbp, 6.6 kbp, 6.0 kbp, 4.2 kbp, 3.8 kbp, 3.6 kbp, 3.1 kbp, dan 2.3 kbp.

Dalam perbandingan serotip yang heterologus, antara serotip A2, A7 dan A9 didapati gelung profil DNA yang terbentuk adalah hampir sama dalam setiap replikasi pengasingan, tetapi masih terdapat sedikit perbezaan. Dalam perbandingan biotip A (serotip A2, A7, A9) dengan biotip T terdapat perbezaan yang agak ketara dimana ciri-ciri gegelung DNA yang istimewa pada serotip T tidak terdapat pada serotip A2, A7 dan A9. Maka disimpulkan ciri imunogen istimewa bagi serotip A2 ialah dengan kehadiran gelung 9.1 kbp dan bagi T pula pada gelung 5.5 kbp, manakala serotip A7 ialah dengan ketiadaan gelung 4.0 kbp, 3.4 kbp dan 2.6 kbp. Bagi serotip A9 pula ketiadaan gelung 2.1 kbp.

Pada keseluruhannya, teknik REA adalah teknik terbaik yang dicadangkan dalam pengenalpastian sekumpulan genotip yang hampir sama kerana penggunaan genomik DNA yang digunakan mengandungi maklumat genetik yang sama dalam semua jenis sel pada organisma tersebut.

# ABSTRACT

The objectives of this study were to evaluate the ability used of the Restriction Endonuclease Analysis (REA) technique for identification and characterization of different serovars of *Pasteurella haemolytica* biotype A and T.

Four replications of *Pasteurella haemolytica* serotype A2, A7, A9, and biotype T were isolated from lung of sheep that were infected by pneumonic pasteurellosis. The DNA isolate of bacteria were extracted by "Wizard® genomic purification kit". REA analysis was done for four replication using *Hae* III restriction endonuclease enzyme. DNA pattern bands then compared by scoring with 6 major band marker (marker LambdaDNA/*Hind* III). Sizes of molecular weight were then determined by plotting a linear curve graph.

REA analysis of homologous serotypes revealed similar resolution of all four replication of isolates in all serovars of *Pasteurella haemolytica* A2, A7, A9 and T. However there are a few identical and common bands presence in all serotypes, the bands were 16.8 kbp, 13.7 kbp, 10.7 kbp, 8.5 kbp, 6.6 kbp, 6.0 kbp, 4.2 kbp, 3.8 kbp, 3.6 kbp, 3.1 kbp, and 2.3 kbp.

comparison of heterologous serotypes A2, A7, A9 and T, showed that the DNA profile band were slightly similar in all replication of isolate. However there still have a differences, in the comparison of biotype A (serotype A2, A7, A9) with T biotype, there are slightly different when the immunogen characteristic and special bands were presence in biotype T, instead absence in serotype A2, A7, and A9. For conclusion, the immonugen characteristic and special bands of serotype A2 are the present of band 9.1 kbp and serotype T the present band of 5.5 kbp. In serotype A7 the characteristic and special DNA band are the absence of band 4.0 kbp, 3.4 kbp and 2.6 kbp. For serotype A9 the absence band of 2.1 kbp.

In conclusion, REA technique was a best preferable technique to identify groups of similar genotype, because the DNA genome used in this study are consists of the similar genetic information in all cell types of the organism.