



UNIVERSITI PUTRA MALAYSIA

***IN VIVO SURVIVALITY AND OPTIMIZATION OF PARAMETERS FOR
BIOMASS PRODUCTION OF GDHA DERIVATIVE OF PASTEURELLA
MULTOCIDA B:2***

SITI NUR HAZWANI BT OSLAN

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BIOMASS PRODUCTION OF *GDHA* DERIVATIVE OF *PASTEURELLA*
MULTOCIDA B:2**

By

SITI NUR HAZWANI BT OSLAN

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

June 2013

Specially dedicated to:

My Family;

HAJI OSLAN ABDUL GHANI

HAJJAH NOOR HAYATI MOHD ZAIN

MOHD. LUKHMAN OSLAN

HAJJAH SITI NURBAYA OSLAN

MOHD. FARIS FIRDAUS OSLAN

MOHD. BAZLI FAHMI OSLAN

MOHD. YATIMI HAKIM OSLAN

My Little Nephew;

MOHD NORMAN DANIEL MOHD. LUKHMAN

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

IN VIVO SURVIVALITY AND OPTIMIZATION OF PARAMETERS FOR BIOMASS PRODUCTION OF *GDHA* DERIVATIVE OF *PASTEURELLA MULTOCIDA* B:2

By

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June 2013

Chairman: Professor Mohd. Zamri Saad, DVM, PhD

Faculty: Veterinary Medicine

The *gdhA* derivative of *Pasteurella multocida* B:2 was earlier created, proven of their safety and was consequently used to control haemorrhagic septicemia in cattle and buffaloes as a vaccine. However, the production cost of the vaccine is too high. This study highlights on low cost medium formulation to mass-produce the vaccine for field use. The survival period of the *gdhA* derivative *Pasteurella multocida* B:2 was studied. In the *in vitro* study, the effect of storage at different temperature revealed that the organism survived better when kept at 4°C compared to room temperature. In the *in vivo* study, the derivative strain attempted to release the kanamycin cassette after 8 h post-intraperitoneal inoculation into mice, but none of the infected mice died. Successful re-isolation of the derivative without cassette from the liver and lung of mice at 88 h post-injection indicated that the strain had significantly reduced the ability to spread and survive *in vivo*. However, following infection by the wild-type *Pasteurella multocida*

B:2, all mice died in less than 24 hours. Subsequently, the wild type was successfully isolated from the heart, lung and liver of mice infected.

Following successful *in vitro* and *in vivo* studies and the earlier studies that indicated the *gdhA* derivative *Pasteurella multocida* B:2 as a good potential for vaccine production. The effects of different nitrogen and carbon sources on biomass production and growth of mutant strain was subsequently studied. The yeast extract and glucose, as nitrogen and carbon source, respectively were proven to help in producing highest biomass. Using the shake-flask, the new growth medium was optimized and developed by Response Surface Methodology (RSM), and produced 3.1 mg/mL of biomass. The RSM suggested that 15.6 g/L of yeast extract, 1.9 g/L of glucose, 3.0 g/L of sodium chloride, and 2.5 g/L of sodium chloride were the optimum amount in the new growth medium to produce optimum product. Biomass production of the mutant strain was tested in a scaled-up 5 L bioreactor. A total volume of 3 L newly formulated medium was used at different oxygen levels. The highest biomass production was effectively at 50% dissolve oxygen, producing 3.50 mg/mL of the of dry cell weight. In conclusion, the improvement in productivity of the mutant *gdhA* derivative of *Pasteurella multocida* B:2 was increased 52.72% in shake-flask and 275.9% in bioreactor before non-optimized medium through RSM method. The total cost of medium components developed in this study (RM7.61 per litre) was low compared with the commercial BHI medium (RM37.00 per litre), which is a reduction of 79%.

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

**KELANGSUNGAN HIDUP *IN VIVO* DAN PARAMETER OPTIMUM UNTUK
PENGELUARAN BIOJISIM MUTAN *GDHA PASTEURELLA MULTOCIDA B: 2***

Oleh

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Mutan *gdhA Pasteurella multocida B:2* telah dicipta, terbukti keselamatan mereka dan seterusnya Berjaya digunakan sebagai vaksin untuk mengawal septisemia berdarah pada lembu dan kerbau. Walaubagaimanapun, kos pengeluaran vaksin tersebut adalah tinggi. Kajian ini menekankan kepada penggubalan formulasi media vaksin dengan kos rendah secara besar-besara untuk kegunaan lapangan. Tempoh kadar kelangsungan hidup mutan *gdhA Pasteurella multocida B: 2* telah Berjaya dikaji. Dalam kajian *in vitro*, kesan daripada penyimpanan pada suhu yang berbeza mendedahkan bahawa kadar kelangsungan hidup organism adalah lebih baik apabila disimpan pada 4°C berbanding pada suhu bilik. Kajian *in vivo*, selepas jam 8 tempoh inokulasi secara intraperitoneal ke dalam tikus, didapati bahawa mutan *gdhA Pasteurella multocida B: 2* tanpa kaset kanamisin telah dikesan, tetapi tiada tikus yang mati. Pengasingan semula mutan tanpa kaset telah berjaya diasingkan daripada hati dan paru-paru tikus pada jam 88 setelah

suntikan menunjukkan bahawa keupayaan untuk menyebarkan dan hidup dalam *in vivo* telah berkurang. Berikutan jangkitan oleh jenis liar *Pasteurella multocida* B: 2, semua tikus mati dalam tempoh kurang daripada 24 jam. Jenis liar telah Berjaya diasingkan daripada jantung, paru-paru dan hati tikus yang dijangkiti.

Dalam kajian awal, kajian *in vitro* dan *in vivo* telah menunjukkan mutan *gdhA Pasteurella multocida* B: 2 berpotensi baik untuk pengeluaran vaksin. Kesan nitrogen yang berbeza dan sumber karbon ke atas pengeluaran biojisim dan pengkulturan mutan *gdhA Pasteurella multocida* B: 2 kemudiannya dikaji. Ekstrak yis dan glukosa, sebagai sumber nitrogen dan karbon, masing-masing telah terbukti untuk membantu dalam menghasilkan biojisim tertinggi. Dalam kelalang goncang, medium pertumbuhan baru telah dioptimumkan dan dibangunkan oleh Kaedah Permukaan Respon (RSM) dengan menghasilkan 3.1 mg/mL biojisim. RSM mencadangkan 15.6 g/L ekstrak yis, 1.9 g/L glukosa, 3.0 g/L natrium klorida, dan 2.5 g/L natrium klorida adalah jumlah optimum bagi medium baru untuk menghasilkan produk optimum. Pengeluaran biojisim mutan telah diuji dalam bioreactor berskala-up 5 L. 3 L medium baru telah digunakan pada tahap oksigen yang berbeza. Pengeluaran biojisim tertinggi berkesan pada 50% oksigen, menghasilkan 3.50 mg/mL berat sel kering. Kesimpulannya, peningkatan produktiviti telah meningkat 52.72% dalam kelalang goncang dan 275.9% dalam bioreactor berbanding medium yang belum dioptimumkan melalui kaedah RSM. Dalam kajian ini, kos keseluruhan media baru adalah rendah (RM7.61 per liter) berbanding media komersil BHI (RM37.00 per liter) dengan pengurangan sebanyak 79%.

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I certify that a Thesis Examination Committee has met on 20 June 2013 to conduct the final examination of Siti Nur Hazwani Oslan on her Master of Science thesis entitled “*In vivo* Survivability and Optimization of Parameters for Biomass Production of *gdha* Derivative of *Pasteurella multocida* B:2” in accordance with Universiti Pertanian Malaysia (Higher Degree) Regulation 1981. The recommends that the candidate the awarded the Master of Science.

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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.



SITI NUR HAZWANI OSLAN

Date: 20 June 2013

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