UNIVERSITI PUTRA MALAYSIA

EFFECT OF MEDIUM FORMULATION AND CULTURE CONDITION ON GROWTH AND PLASMID STABILITY OF RECOMBINANT *LACTOCOCCUS LACTIS* AM3

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By

HO HOOI LING

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

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Specially dedicated to my beloved Father, mother, brothers and Willson,

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

EFFECT OF MEDIUM FORMULATION AND CULTURE CONDITION ON GROWTH AND PLASMID STABILITY OF RECOMBINANT *LACTOCOCCUS LACTIS* AM3

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October 2002

Chairman: Hirzun bin Yusof, Ph.D.

Faculty: Food Science and Biotechnology

Chicken Anemia Virus (CAV) is a small spherical negative single stranded DNA virus which comprised of several overlapping open reading frames (ORFs), three of which encoded for the proteins size of 52, 24 and 13 kDa, designated as VP1, VP2 and VP3, respectively. The recombinant *Lactococcus lactis* AM3 used in the study was transformed with a plasmid expression vector, pMG36e, which was cloned with the VP3 gene of CAV. This recombinant *Lactococcus lactis* AM3 was constructed as a model to study the possibility of vaccine delivery into the poultry via oral route. The investigation on the growth of the recombinant *Lactococcus lactis* AM3 in different carbon sources (glucose, sucrose, lactose and xylose) showed that

glucose was the most suitable carbon source where it successfully produced 36 generation numbers of bacteria with final plasmid-bearing cells, 1.13 X 10^{17} cfu.L^{-1} after 24 hours of fermentation. The final plasmid stability of recombinant *Lactococcus lactis* AM3 grew in the glucose medium was 98% indicating that most of the recombinant bacterial still retained their plasmids. There was a stable and optimum growth of the recombinant *Lactococcus lactis* AM3 in the culture medium with 10 g.L^{-1} of glucose concentration. The maximum specific growth rate of plasmid-bearing cells in the culture, μ_{MAXpb} was 0.9885 h^{-1}. The higher concentration of glucose (20, 40 and 60 g.L^{-1}) in the medium inhibited the growth of recombinant *Lactococcus lactis* AM3. The specific growth rate of *Lactococcus lactis* AM3 grew in the medium with 20 g.L^{-1} of yeast extract was the highest, 0.8869 h^{-1} with 84% of plasmid stability. Similar polypeptides average molecular weights are observed in the yeast extract medium that increased the nitrogen consumption by the recombinant bacteria and eventually increased the bacteria cell number.

Further experiments confirmed that 20 g.L^{-1} of glucose and 20 g.L^{-1} of yeast extract containing medium was the optimum carbon-nitrogen source for the growth and plasmid maintenance of the recombinant *Lactococcus lactis* AM3. The specific growth rate and plasmid stability of *Lactococcus lactis* AM3 in this complex medium were increased to 1.6304 h^{-1} and 87%, respectively after 26 hours of batch fermentation. There was no nitrogen deficiency occurred and the lost of plasmid, pMG36e-VP3 from the host cell was caused majority by the segregational instability of plasmid after prolonged fermentation.

The specific growth rate of recombinant *Lactococcus lactis* AM3 grew at pH medium 6.5 was the highest, 1.0227 h^{-1}. 91% of plasmid stability of recombinant *Lactococcus lactis* AM3 grew at pH 6.5 was observed. The maximum specific growth rate of *Lactococcus lactis* AM3 grew at 30°C was 0.9780 h^{-1} with total plasmid-bearing cells of 9.80 X 10^{20} cfu.L^{-1} after 28 hours of fermentation. Similar acid-inducible proteins were produced by recombinant *Lactococcus lactis* AM3 when confronted with high temperatures (32, 34, 36 and 38°C). 90% of plasmid stability was observed in *Lactococcus lactis* AM3 grew at 30°C. Recombinant *Lactococcus lactis* AM3 grew in the MRS medium that agitated with 200 r.p.m. produced 32 generations of plasmid-bearing cells, 3.87 X 10^{19} cfu.L^{-1} with 51% of plasmid stability after 26 hours of fermentation.

Obvious fluctuation of the plasmid stability of pMG36e-VP3-bearing cells was observed during the batch fermentation system. The appearance of adaptive descendants without plasmids derived from the bacterial host cells was detected throughout the experiments. The lost of plasmid, pMG36e-VP3 from recombinant *Lactococcus lactis* AM3 and the fluctuations in the population of plasmid-losing cells in the batch fermentation system suggested that the presence of antibiotic-degraded proteins or acids were produced from the bacterial host cells that responsible in the inhibition of proper function of erythromycin in the culture. Most of the plasmid instability was caused by segregational instability.

The study concluded that the optimum medium composition and growth condition of recombinant *Lactococcus lactis* AM3 were 20 $g.L^{-1}$ of glucose and 20 $g.L^{-1}$ with agitation speed of 200 r.p.m.

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

KESAN MENGGUNAKAN PELBAGAI FORMULASI MEDIA DAN KEADAAN PENGKULTURAN YANG BERBEZA-BEZA KE ATAS TUMBESARAN DAN KESTABILAN PLASMID REKOMBINAN *LACTOCOCCUS LACTIS* AM3

Oleh

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Oktober 2002

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Virus Chicken Anemia (CAV) adalah sejenis virus kecil berbentuk sfera yang mempunyai rantaian DNA negatif tunggal. Rantaian-rantaian DNA virus itu terdiri daripada beberapa bingkai DNA yang terbuka (ORFs), yang berlipat-lipat di atas antara satu sama lain. Tiga daripadanya mengekodkan protein-protein yang bersaiz 52, 24 dan 13 kDa yang digelar sebagai VP1, VP2 dan VP3. Rekombinan *Lactococcus lactis* AM3 yang digunakan di dalam ujikaji ini telah ditransformasikan dengan satu plasmid ekspresi, pMG36e yang diklonkan dengan gen VP3 daripada CAV. Rekombinan bakteria ini telah dihasilkan bertujuan untuk menjadikannya satu

model pengajian dalam penggunaan vaksin ternakan yang disalurkan melalui mulut. Ujikaji-ujikaji ke atas tumbesaran rekombinan bakteria ini dalam pelbagai sumber media karbon (glukosa, sukrosa, laktosa dan xilosa) menunjukkan glukosa adalah sumber karbon yang paling sesuai di mana ia telah menghasilkan 36 generasi bakteria dengan anggaran bilangan sel bakteria berplasmid sebanyak 1.13 X 10^{17} cfu.L^{-1} dalam masa 24 jam pengkulturan. Kestabilan plasmid bagi rekombinan bakteria ini dalam glukosa media adalah 98% menunjukkan kebanyakan rekombinan sel bakteria mengekalkan plasmid mereka. Terdapat tumbesaran rekombinan bakteria *Lactococcus lactis* AM3 yang stabil dan optima di dalam kultur media yang kepekatan glukosa, 10 g.L^{-1}. Kadar tumbesaran spesifik bagi sel bakteria berplasmid di dalam kultur itu adalah 0.9885 h^{-1}. Kepekatan glukosa yang lebih tinggi di dalam media (20, 40 dan 60 g.L^{-1}) akan menghalang tumbesaran *Lactococcus lactis* AM3. Kadar tumbesaran spesifik bagi *Lactococcus lactis* AM3 di dalam kultur media yang mengandungi 20 g.L^{-1} ekstrakan yis adalah tertinggi, 0.9885 h^{-1} dengan kestabilan plasmid sebanyak 84%. Purata berat molekul-molekul polypeptida adalah sama di dalam media yang mengandungi ekstrakan yis. Ia menambahkan penggunaan sumber nitrogen oleh rekombinan *Lactococcus lactis* AM3 dan seterusnya meningkatkan bilangan sel bakteria di dalam kulturnya.

Kajian-kajian seterusnya telah mengesahkan bahawa 20 g.L^{-1} glukosa dan 20 g.L^{-1} ekstrakan yis merupakan media karbon dan nitrogen yang optima untuk tumbesaran dan pengekalan plasmid di dalam rekombinan *Lactococcus lactis* AM3. Kadar tumbesaran spesifik dan kestabilan plasmid *Lactococcus lactis* AM3 yang

hidup di dalam media itu telah meningkat ke 1.6304 h^{-1} dan 87%, masing-masing dalam 26 jam pengulturan. Tidak terdapat penggunaan nitrogen yang membazir berlaku dan kehilangan plasmid, pMG36e-VP3 daripada sel hos bakteria adalah disebabkan oleh ketidakstabilan segregasi plasmid yang berlaku di dalam kultur bakteria ini.

Kadar tumbesaran spesifik *Lactococcus lactis* AM3 yang hidup pada pH 6.5 merupakan yang tertinggi iaitu 1.0227 h^{-1}. 91% kestabilan plasmid bagi rekombinan *Lactococcus lactis* AM3 yang hidup pada pH 6.5 telah diperolehi. *Lactococcus lactis* AM3 yang hidup pada suhu 30°C itu telah menghasilkan kadar tumbesaran spesifik yang tertinggi iaitu, 0.9780 h^{-1} dengan anggaran bilangan sel bakteria berplasmid sebanyak 9.80 X 10^{20} cfu.L^{-1} dalam masa 28 jam pengkulturan. Protein-protein cetusan asid juga yang dihasilkan oleh *Lactococcus lactis* AM3 apabila mereka berhadapan dengan suhu yang tinggi (32, 34, 36 dan 38°C). 90% kestabilan plasmid bagi rekombinan *Lactococcus lactis* AM3 yang hidup pada suhu 30°C telah diperhatikan. *Lactococcus lactis* AM3 yang hidup pada putaran 200 rpm berjaya menghasilkan 32 generasi bakteria dengan bilangan sel bakteria berplasmid sebanyak 3.87 X 10^{19} cfu.L^{-1} dan sebanyak 51% populasi sel bakteria yang mengekalkan plasmid mereka.

Ketidakstabilan plasmid bagi sel bakteria berplasmid pMG36e-VP3 dapat diperhatikan di dalam fermentasi berkelompok. Kewujudan generasi sel yang tidak berplasmid daripada hos sel bakteria diperhatikan di sepanjang ujikaji-ujikaji ini.

Ketidakstabilan plasmid yang berlaku disebabkan oleh ketidakstabilan segregasi tetap wujud walaupun antibiotik eritromisin telah digunakan sebagai media pemilihan. Kewujudan protein atau asid telah menghancurkan antibiotik dan seterusnya menyebabkan kehilangan plasmid. Antibiotik yang dihasilkan oleh hos sel bakteria bertanggungjawab menghalang fungsi eritromisin bertindak dengan baik di dalam kultur bakteria. Sesetengah sel bakteria tidak berplasmid dihasilkan melalui proses mutasi yang berlaku di dalam kromosom bakteria. Ia menghasilkan kadar tumbesaran spesifik yang lebih tinggi bagi sel bakteria tidak berplasmid. Kebanyakan ketidakstabilan plasmid disebabkan oleh ketidakstabilan segregasi kerana tidak terdapat masa yang mencukupi untuk replikasi plasmid DNA diturunkan kepada generasi sel yang berterusan. Bilangan rendah plasmid adalah sebab utama kehilangan plasmid melalui ketidakstabilan segregasi.

Ujikaji-ujikaji ini telah menghasilkan formulasi media dan keadaan pengkulturan yang optima bagi rekombinan *Lactococcus lactis* AM3 iaitu ia hidup dalam 20 $g.L^{-1}$ glukosa dan 20 $g.L^{-1}$ ekstrakan yis pada pH 6.5, suhu, 30°C dengan putaran, 200 r.p.m. dalam sistem fermentasi.

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I certify that an Examination Committee met on the 21st October 2002 to conduct the final examination of Ho Hooi Ling on her Master Science thesis entitled "Effect of Medium Formulation and Culture Condition on Growth and Plasmid Stability of Recombinant *Lactococcus lactis* AM3" in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the candidate be awarded the relevant degree. Members of the Examination Committee are as follows:

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The thesis submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfillment of the requirement for the degree of Master Science. The members of the Supervisory Committee are as follow:

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DECLARATION FORM

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 currently submitted for any other degree at UPM or other institutions.

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