
**UNIVERSITI PUTRA MALAYSIA**

# *PROBIOTIC POTENTIAL OF AND CONJUGATED LINOLEIC ACID PRODUCTION BY BACTERIA ISOLATED FROM CHICKEN AND RUMINANTS*

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# PROBIOTIC POTENTIAL OF AND CONJUGATED LINOLEIC ACID PRODUCTION BY BACTERIA ISOLATED FROM CHICKEN AND RUMINANTS

**By**

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Conjugated linoleic acid (CLA) refers to fatty acids with 18-carbon and 2 conjugated double bonds in different positional and geometric configurations (C18:2). CLA can be normally found in ruminant meat, milk, cheese, dairy products, egg yolk and chicken. Dietary CLA intake by human is too low to exhibit health benefits such as anti-cancer, anti-inflammatory, anti-atherosclerosis, anti-obesity and modulation of immune system. Furthermore, the purity of CLA isomers is crucial for human health but the commercially available CLA was mostly produced by chemical synthesis which leads to the production of mix isomers of CLA. This can be achieved through bioproduction of CLA by bacteria and the increase of consumption of CLA enriched products by human. Therefore, the objectives of this study were to investigate the ability of bacteria isolated from ruminants, chicken, human milk and infant feces to produce CLA and to evaluate their probiotic characteristics and factors that affect the CLA production. In this study, 120 isolates were tested for the presence of catalase and Gram staining. A total of 69 isolates with catalase negative, Gram positive and

grown on MRS medium under anaerobic condition were screened for CLA production. From the screening results, 18 isolates which isolated from cattle, deer and chicken have the ability to produce CLA from lactic acid (LA). The four highest CLA-producing bacteria were isolated from chicken and cattle. They were identified as *Lactobacillus salivarius* strain P2, *Lactobacillus agilis* strain P3, *Enterococcus faecium* strain P1 and *Streptococcus equinus* strain C3 based on molecular method and produced CLA concentration of 21.97, 31.08, 23.35 and 10.23 µg/ml, respectively. *Lactobacillus salivarius* strain P2, *L. agilis* strain P3, *E. faecium* strain P1 and *S. equinus* strain C3 consist of 60.65%, 66.90%, 49.77% and 52.01% of *cis*-9, *trans*-11 (*c*9, *t*11) CLA isomer and 39.35%, 33.10%, 50.23% and 47.99% of *trans*-10, *cis*-12 (*t*10, *c*12) CLA isomer, respectively. Reference strain, *Lactobacillus reuteri* ATCC 55739 produced CLA concentration of 122.45 µg/ml which consist of 89.24% of *c*9, *t*11 CLA isomer and 10.76% of *t*10, *c*12 CLA isomer. All bacteria tested were able to tolerate 0.3 % bile salts and pH 2.5 acidic condition. As compared to *L. reuteri* ATCC 55739, *L. agilis* strain P3 and *L. salivarius* strain P2 showed better acidic tolerance, resistant to two types of antibiotics tested, higher antimicrobial activity and ability to produce higher amount of lactic acid. All bacterial strains showed no bacteriocin production. *Streptococcus equinus* strain C3 was resistant to four types of antibiotic tested and *E. faecium* strain P1 was hemolytic positive bacteria. These characteristics have made these 2 strains not suitable as probiotic bacteria. The most efficient CLA production were obtained by 20% cell density in citrate buffer, pH 5.5 containing 1.0 mg/ml LA which were incubated at 30°C for 48 h with shaking at 120 rpm under aerobic condition. Under optimal reaction conditions, *L. reuteri* ATCC 55739 produced the highest concentration of CLA, 280.75 µg/ml reaction mixture, followed by *L. agilis* strain P3 with 109.78 µg/ml

reaction mixture and *L. salivarius* strain P2 with 84.58 µg/ml reaction mixture. The CLA isomers formed were majority *c*9, *t*11 CLA and minority *t*10, *c*12 CLA. Taking into account of the probiotic effect of bacteria, *L. salivarius* strain P2 and *L. agilis* strain P3 are more suitable to be probiotic for animals and to produce CLA extracellular. The findings in this study prompt further studies to be carried out to investigate the ability of bacteria to produce CLA and their probiotic effects in animal model.

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

**POTENSI PROBIOTIK DAN PENGHASILAN ASID LINOLEIK TERKONJUGAT OLEH BAKTERIA YANG DIPENCILKAN DARIPADA AYAM DAN RUMINAN**

Oleh

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Asid linoleik terkonjugat (CLA) merujuk kepada asid lemak dengan 18 karbon dan 2 ikatan ganda dua terkonjugat dalam konfigurasi kedudukan dan geometri yang berbeza (C18:2). CLA biasanya boleh ditemui dalam daging ruminan, susu, keju, produk tenusu, kuning telur dan ayam. Pengambilan CLA dalam diet oleh manusia adalah terlalu rendah untuk menunjukkan manfaat kesihatan seperti anti-kanser, anti-radang, anti-aterosklerosis, anti-obesiti dan modulasi sistem imun. Tambahan pula, ketulenan CLA isomer adalah penting untuk kesihatan manusia tetapi kebanyakan CLA yang didapati secara komersial adalah dihasilkan daripada sintesis kimia di mana sintesis ini membawa kepada penghasilan isomer campuran CLA. Ini boleh dicapai melalui penghasilan biologi CLA oleh bakteria dan peningkatan pemakanan produk yang diperkaya dengan CLA oleh manusia. Oleh itu, objektif kajian ini adalah untuk menyelidik keupayaan bakteria yang dipencilkan daripada ruminan, ayam, susu manusia dan najis bayi untuk menghasilkan CLA dan untuk menilai ciri-ciri probiotik bakteria dan faktor-faktor yang mempengaruhi pengeluaran CLA.

Dalam kajian ini, 120 isolat telah diuji untuk kehadiran katalase dan pewarnaan Gram. Sebanyak 69 isolat dengan katalase negatif, Gram positif dan tumbuh atas medium MRS di bawah keadaan anaerobik telah disaring untuk penghasilan CLA. Daripada keputusan penyaringan, 18 isolat yang dipencilkan daripada lembu, rusa dan ayam mempunyai keupayaan untuk menghasilkan CLA daripada asid laktik (LA). Empat bakteria yang mempunyai penghasilan CLA yang tertinggi adalah dipencilkan daripada ayam dan lembu. Bakteria telah dikenalpasti sebagai strain P2 *Lactobacillus salivarius*, strain P3 *Lactobacillus agilis*, strain P1 *Enterococcus faecium* dan strain C3 *Streptococcus equinus* berdasarkan cara molekul dan menghasilkan kepekatan CLA dengan 21.97, 31.08, 23.35 dan 10.23 µg/ml, masing-masing. Strain P2 *Lactobacillus salivarius*, strain P3 *L. agilis*, strain P1 *E. faecium* dan strain C3 *S. equines* terdiri daripada 60.65%, 66.90%, 49.77% dan 52.01% isomer CLA *cis*-9, *trans*-11 (*c*9, *t*11) dan 39.35%, 33.10%, 50.23% dan 47.99% isomer CLA *trans*-10, *cis*-12 (*t*10, *c*12), masing-masing. Strain rujukan, *Lactobacillus reuteri* ATCC 55739 menghasilkan kepekatan CLA dengan 122.45 µg/ml yang terdiri daripada 89.24% isomer CLA *c*9, *t*11 dan 10.76% isomer CLA *t*10, *c*12. Semua bakteria yang diuji boleh toleransi kepada 0.3% garam hempedu dan keadaan asid pH 2.5. Jika berbanding dengan *L. reuteri* ATCC 55739, strain P3 *L. agilis* dan strain P2 *L. salivarius* menunjukkan toleransi asid yang lebih baik pada pH 2.5, rintangan terhadap dua jenis antibiotic yang diuji, aktiviti antimikrob yang lebih tinggi dan keupayaan untuk menghasilkan jumlah asid laktik yang lebih tinggi. Semua strain bakteria menunjukkan tiada penghasilan bakteriosin. Strain C3 *Streptococcus equines* mempunyai rintangan terhadap empat jenis antibiotik yang diuji dan strain P1 *E. faecium* menunjukkan hemolitik positif. Ciri-ciri ini telah menjadikan 2 strain ini tidak sesuai sebagai bakteria probiotik. Penghasilan CLA

yang paling efisien diperolehi dengan 20% kepadatan sel dalam penimbal sitrat, pH 5.5 yang mengandungi 1.0 mg/ml LA yang dieram pada 30°C selama 48 jam dengan 120 rpm di bawah keadaan aerobik. Di bawah keadaan reaksi yang optimum, *L. reuteri* ATCC 55739 menghasilkan kepekatan CLA yang tertinggi, 280.75 µg/ml campuran, diikuti oleh strain P3 *L.agilis* dengan 109.78 µg/ml campuran dan strain P2 *L. salivarius* dengan 84.58 µg/ml campuran. Isomer CLA yang terbentuk majoriti adalah *c*9, *t*11 CLA dan minoriti adalah *t*10, *c*12 CLA. Jika kesan probiotik diambil kira, strain P2 *Lactobacillus salivarius* dan strain P3 *L. agilis* adalah lebih sesuai untuk dijadikan sebagai probiotik untuk haiwan dan menghasilkan CLA ekstrasellular. Penemuan dalam kajian ini mewajarkan kajian lebih lanjut dijalankan untuk menyiasat keupayaan bakteria menghasilkan CLA dan kesan probiotik dalam model haiwan.

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I certify that a Thesis Examination Committee has met on 23 July 2013 to conduct the final examination of Yong Su Ting on her thesis entitled "Probiotic Potential of and Conjugated Linoleic Acid Production by Bacteria Isolated from Chicken and Ruminants" in accordance with the Universities and University College 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 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 other institutions.

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**YONG SU TING**

Date: 23 July 2013

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