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ISOLATION AND EVALUATION OF PROBIOTIC POTENTIAL OF LACTIC ACID BACTERIA IN VILLAGE CHICKEN GASTROINTESTINAL TRACT

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By

ARLENE DEBBIE ANAK LINGOH

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ISOLATION AND EVALUATION OF PROBIOTIC POTENTIAL OF LACTIC ACID BACTERIA IN VILLAGE CHICKEN GASTROINTESTINAL TRACT

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May 2020

Chair : Leong Sui Sien, PhD
Faculty : Agriculture and Food Science (Bintulu Campus)

Currently, small-scale poultry industries are still utilising feed-additive antibiotics in livestock growth improvement and feed efficiency although it has been banned due to the increased incidences of antibiotic resistance among zoonotic bacteria and the loss of therapeutic efficacy in both veterinary and human medicine. A group of lactic acid bacteria in probiotic, which is a live beneficial microbial feed supplement has been used in commercial animal feed as substitution to the antibiotic. However, the continuous increase in demand for organic farming in food-producing animals clearly necessitate probiotic strains with more effective properties to reduce diseases and improve productivity. This study was conducted purposely to identify and characterize potential probiotic properties and diversity of lactic acid bacteria isolated from the intestinal tract of village chicken. Village chickens that have not been fed any commercial feed have been used as a source of potential probiotic strains to prevent a source of antibiotic-resistant lactic acid bacteria. Five village chickens were caught randomly from every four locations around Bintulu to collect the crop, gizzard, small intestine, large intestine and cecum. Samples were processed and plated for bacterial colony count. 800 bacteria were isolated, clustered and analysed using $(GTG)_5$ fingerprinting and probiotic analysis test. Phenotypic characterization resulted in only 21 isolates possess the lactic acid bacteria characteristics such as gram-positive, catalase-negative, non-motile and able to ferment glucose. Genotypic characterization has identified that the isolated lactic acid bacteria was *Enterococcus*, *Lactobacillus* and *Bacillus*. Only isolates CR1AM6, L1M10, C4M8, L4AM2 and L4AM5 has shown resistant towards bile salt, pepsin and acidic condition which will enable these strains to survive the harsh condition in the gut. These isolates also able to ferment inulin which is beneficial towards the host as it helps the live microbiota to be implemented easily in the gut.

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

PENGASINGAN DAN PENILAIAN POTENSI PROBIOTIK BAKTERIA ASID LAKTIK DALAM SALURAN GASTROUSUS AYAM KAMPUNG

Oleh

ARLENE DEBBIE ANAK LINGOH

Mei 2020

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Pada masa ini, industri unggas berskala kecil masih menggunakan antibiotik aditif makanan dalam peningkatan pertumbuhan ternakan dan kecekapan pakan walaupun telah dilarang kerana peningkatan kejadian ketahanan terhadap antibiotik di kalangan bakteria zoonotik dan hilangnya keberkesanan terapi dalam perubatan veterinar dan manusia. Sekumpulan bakteria asid laktik dalam probiotik, yang merupakan makanan tambahan mikroba bermanfaat hidup telah digunakan dalam makanan haiwan komersial sebagai pengganti antibiotik. Namun, peningkatan permintaan pertanian organik pada haiwan penghasil makanan secara berterusan memerlukan strain probiotik dengan sifat yang lebih berkesan untuk mengurangkan penyakit dan meningkatkan produktiviti. Kajian ini dilakukan dengan sengaja untuk mengenal pasti dan mencirikan potensi probiotik dan kepelbagaian bakteria asid laktik yang diasingkan dari saluran usus ayam kampung. Ayam kampung yang tidak diberi makanan komersial telah digunakan sebagai sumber strain probiotik yang berpotensi untuk mencegah sumber bakteria asid laktik tahan antibiotik. Lima ekor ayam kampung ditangkap secara rawak dari setiap empat lokasi di sekitar Bintulu untuk mengumpulkan tanaman, gizzard, usus kecil, usus besar dan cecum. Sampel diproses dan dilapisi untuk penghitungan koloni bakteria. 800 bakteria diasingkan, dikelompokkan dan dianalisis menggunakan $(GTG)_5$ cap jari dan ujian analisis probiotik. Pencirian fenotipik mengakibatkan hanya 21 isolat yang mempunyai ciri bakteria asid laktik seperti gram positif, katalase-negatif, tidak bergerak dan mampu menetap glukosa. Pencirian genotip telah mengenal pasti bahawa bakteria asid laktik terpencil adalah *Enterococcus*, *Lactobacillus* dan *Bacillus*. Hanya pencilan CR1AM6, L1M10, C4M8, L4AM2 dan L4AM5 yang menunjukkan rintangan terhadap garam hempedu, pepsin dan keadaan berasid yang akan membolehkan pencilan ini bertahan dalam keadaan yang extrem di usus. Pencilan ini juga dapat memfermentasi inulin yang bermanfaat bagi mikrobiota kerana ia membantu mikrobiota hidup dengan mudah di dalam usus.

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I certify that a Thesis Examination Committee has met on 19 May 2020 to conduct the final examination of Arlene Debbie anak Lingoh on her thesis entitled "Isolation and Evaluation of Probiotic Potential of Lactic Acid Bacteria in Village Chicken Gastrointestinal Tract" in accordance with the Universities and University Colleges 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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This is to confirm that:

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Signature: ______________________

Name of Member of Supervisory Committee: Assoc. Prof. Dr. Shahrul Razid Sarbini

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBRIEVIATIONS

HPAI	Highly Pathogenic Avian Influenza
US	United States
EU	European Union
FAO	Food and Agriculture Organisation
AGP	Antibiotic Growth Promoters
LAB	Lactic Acid Bacteria
FAOSTAT	Food and Agriculture Organization Corporate Statistical Database
FDA	Food and Drug Administration
USA	United States of America
WHO	World Health Organization
FAO/WHO	Food and Agriculture Organization of the United Nations and the World Health Organisation
GIT	Gastrointestinal Tract
GB	Goblet cells
IELs	Intraepithelial lymphocytes
PCR	Polymerase Chain Reaction
DNA	Deoxyribonucleic Acid
dNTPs	deoxyribonucleotide triphosphates
rDNA	Recombinant Deoxyribonucleic Acid
UPMKB	Universiti Putera Malaysia Kampus Bintulu
PBS	Phosphate Buffer Saline
MRS	de-Mann Rogosa Sharpe
NB	Nutrient broth
NA	Nutrient Agar
EMB	Eosin Methylene Blue

CFU	Colony Forming Unit
NCBI	National Center for Biotechnology Information
UK	United Kingdom
OD	Optical density
SSF	Simulated Salivary Fluid
SIF	Simulated Intestinal Fluid
SGF	Simulated Gastric Fluid
TAE	Tris acetate EDTA
KCl	Potassium Chloride
KH_2PO_4	potassium dihydrogenphosphate
$NaHCO_3$	Sodium bicarbonate
NaCl	Sodium Chloride
$MgCl_2.6H_2O$	Magnesium Chloride, Hexahydrate
$(NH_4)2CO_3$	Ammonium carbonate
$CaCl_2.2H_2O$	Calcium Chloride Dihydrate
HCl	Hydrochloric Acid
NaOH	Sodium Hydroxide
Cinh	Coefficient of inhibition
C	Cecum
CR	Crop
S	Small intestine
L	Large intestine
G	Gizzard
CO_2	Carbon dioxide
BSH	bile salt hydrolase enzyme
H_2O_2	Hydrogen peroxide

SCFA	Short chain fatty acid
DP	Degree of polymerization

CHAPTER 1

INTRODUCTION

Recently, domesticated bird's flesh and eggs are the foremost consumed food derived from animal amongst different sources world widely and it became vital for food security and nutrition. Because of its natural origin, ability to provide the essential macronutrient and meet the global needs, the industry arises as to the foremost competent sub-sector (Mottet & Tempio, 2017). The most widespread in poultry meat such as turkey, duck, hens and chicken is 70 to 80 percent of world consumption, as stated by Skarp *et al*., (2016).The world's preferred source of protein can be considered chicken meat because it's cheap and easy to get. For the last 50 years, compared to other livestock products, the average annual growth for poultry was 5% while it was only 1.5%, 3.1% and 1.7% for beef, pork and ruminants respectively (Alexandratos & Bruinsma, 2012). World production of poultry meat in 2017 is forecast at 120.5 million, up 1.1 per cent from 2016. Following regular outbreaks of Highly Pathogenic Avian Influenza (HPAI) in Africa, Asia and Europe, production increased in almost all major poultry growing areas. Most of those increased production in 2017 originated from the US (+2.4%), the Russian Federation (+7.2%), Brazil (+1.9%), Turkey (+12.5%), India (+4.8%), Thailand (+6.7%), Mexico (+3.8%), and the EU (+0.8%). World poultry meat demand increased by 21.3 million tonnes between 2010 and 2017 (Food and Agriculture Organization, 2018). According to Mottet & Tempio, (2017), due to rising population and consumer incomes, global demand for cheaper protein sources will continue to intensify with the increase in global chicken consumption. This will lead to an increase in the production of commercial chicken to meet global demand.

In Malaysia, the establishment of a reliable and safe poultry-specialized system has allowed entrepreneurs to enhance their poultry business. According to the 2018 Department of Veterinary Services Malaysia survey, the production of poultry meat rises from 1,664.9('000 M. Tonne) in 2017 to 1,653.7('000 M. Tonne) in 2018. In addition, the number is expected to rise into 1,716.7('000 M. Tonne) in 2019 (Department of Veterinary Services, 2018). This improvement has modified the Malaysian poultry industry from the smaller grounds production system to extremely modernised, commercialised and economical production systems. The high consumption has helped the Malaysian poultry industry to support the high production output throughout the years compared to other livestock industries. Besides being the most cost-effective supply of meat protein, chicken meat has no prohibitions or restrictions in dietary and religion consumption in contrast with pork and beef that are restricted to Malays and Hindus, correspondingly. Therefore, the availability consumption of the meat to any or all races conjointly contributed to the rapid climb of the industry (Abdurofi *et al*., 2017). However, the increasing demand has raised concern for the long-run sustainability of the poultry industry. As a result, most poultry farmers resulted within the use of antibiotics to increase growth rates, manufacture more eggs, and reduce death rate and diseases (Samsuddin *et al*., 2015).

One of the foremost exceptional medical uncovering of 20th century is the discovery of antibiotics while their functions in treating the animal and human diseases are still crucial during this 21st century. Between the early 1940s to 1950s, antibiotics have been widely used in the animal food industry (Allen *et al*., 2013). In animal production, antibiotics are being used for medicine treatments, prophylactics preventatives, and growth starters in food animal production (Cheng *et al*., 2014). They are even have been enforced in animal feed as a growth promoter in expanding feed potency of conventional farm animal and poultry production (Allen *et al*., 2013; Mungroo & Neethirajan, 2014). Growth promoters is defined as the substances that are deliberately supplemented to a diet to trigger the utmost response of the genomic potential of the host in terms of development and enhancement in feed effectiveness (Dhama *et al*., 2014). However, the long use and the misuse of antibiotics as a growth promoter have increased antibiotic-resistant microorganism and this has turned the meat chicken as the medium for the transmission of multidrug-resistant microorganism to the consumers (Salim *et al*., 2013; Aliyu *et al.*,. As a result, this could cause the failure of antibiotic treatment (Salim *et al*., 2013; Mungroo & Neethirajan, 2014) and may be impossible to beat infections (Mungroo & Neethirajan, 2014). All the reported cases show that the continual of exploitation antibiotics in feed animals have led to serious issues as they can threaten public health and reduces drug effectiveness. Therefore, the use of antibiotics in animal feed has been forbidden by many countries including South Korea, which has explicitly restricted the use of antibiotic growth promoters (AGP) in the animal diet (Salim *et al*., 2013). With the elimination of antibiotics as a growth driver for livestock, the poultry industry is now facing a major challenge in sustaining output efficiency, as feed prices have risen and the prohibition on antimicrobial usage in feed causes high morbidity and mortality in poorly managed flocks (Salim *et al*., 2013; Dhama *et al*., 2014). Efficient substitution of growth promoters is therefore urgently needed to ensure that contemporary levels of animal production can be maintained without intimidating public health (Seal *et al.* 2013).

One of the solutions that are accustomed overcome the antibiotic-resistant in animal feed is by substituting antibiotics with probiotics. Recently, probiotics have gained a lot of interest from the broiler chicken industry as a substitution for the prophylactic usage of antibiotics in animal feeds (Musikasang *et al.*, 2009; Salim *et al*., 2013). According to Hill *et al*., (2014), the definition of probiotic is "live microorganisms that, when administered in adequate amounts, confer a health benefit on the host". Due to historical belief that lactic acid bacteria are necessary members of the colonic microflora and consequently commonly regarded as safe, it often used in most probiotics preparation (Jadhav *et al*., 2018). The feeding of chicken with diet supplemented with probiotics will benefit the birds by having a balance ecological microflora inside the gut and minimizing the growth of potential pathogens like enterobacteria and Campylobacter species in poultry gut which will cause damage to the host by enhancing their immunity system (Aliakbarpour *et al*., 2012; Ritzi, *et al*., 2018).

Although numerous publications show improvement in the performance of broilers, layers and turkeys, there are also reports that probiotics have a limited and variable growth-promoting effect and, in some cases, none (Karaoglu and Durdag, 2005; O'Dea *et al*., 2006; Lee *et al*., 2010; Waititu *et al*., 2014). This inconsistency in results can be attributed to differences in the type and dose of strain used, variations in treatment, duration and duration of administration, diet and the environment. Various commercial probiotic products on the global market are currently available for poultry. Nonetheless, due to inadequate analysis of the unique beneficial properties of the probiotic strains formulated in the drug, some of them may not be highly potent (Binek, 2016). Moreover, most manufacturers lack the patience to carry out an in-depth study of each strain in order to ascertain its full probiotic potential before commercialization, as most industries maximize profits at minimal cost. (Reuben *et al*., 2019). While several LAB strains have probiotic characteristics and have been established as suitable probiotic supplements for different hosts, new probiotic strains with better health promotion characteristics are still being searched for than are currently available in the market. Consequently, this research was designed to isolate, identify and test strains of lactic acid bacteria with sufficient probiotic properties from the village chicken gut for use as poultry probiotics.

The objectives of the study are:

1. To isolate lactic acid bacteria from different part of the gastrointestinal tract of village chicken.
2. To evaluate the probiotic potential of lactic acid bacteria isolated from different part of gastrointestinal tract of village chicken.
3. To investigate the effect of inulin on the growth of lactic acid bacteria.

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