EFFECTS OF *LACTOBACILLUS PLANTARUM* DR7 ON STRESS AND ANXIETY IN ADULTS: A RANDOMISED, DOUBLE-BLIND, PLACEBO-CONTROLLED STUDY

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by

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5-HT_6	5-hydroxytryptamine receptor-6
5-HT	Serotonin
ACTH	Adrenocorticotropic hormone
AMPK	Adenosine monophosphate-activated protein kinase
ANS	Autonomic nervous system
API	Analytic profile index
ATCC	American Type Collection Centre
ATP	Adenosine triphosphate
BDI	Beck depression inventory
BDNF	Brain-neurotrophic factor
BMI	Body Mass Index
CBB	CogState Brief Battery
CDC	Centers for Disease Control and Prevention
CES-D	Center for epidemiological studies depression scale
CFS	Cell-free supernatant
CFU	Colony form unit
CNS	Central nervous system
CREB	cAMP-response element binding protein
CRF	Corticotropin releasing factor
CRH	Corticotropin releasing hormone
Ct	Threshold cycle
DA	Dopamine
DBH-Dep	Dopamine β-hydroxylase
DMEM	Dulbecco's modified essential medium DNA
DNA	Deoxyribonucleic acid
DR7	*Lactobacillus plantarum* DR7
EFSA	European Food Safety Authority
ELISA	Enzyme-linked immunosorbent assay GOS
ENS	Enteric nervous system
FAO	Food and Agriculture Organization FOS

FDA	Food and Drug Administration
FOS	Fructooligosaccharide
FRAP	Ferric reducing ability
GABA	γ-aminobutyric acid
GABRA5	Gamma aminobutyric acid A-receptor α-5
GAD65	Glutamic acid decarboxylase 65
GBOD	Global Burden of Disease Study
GDS-SF	Geriatric depression scale-short form
GF	Germ-free
GHQ-28	28-item General health questionnaire
GI	Gastrointestinal
GMP	Good Manufacturing Practice
GOS	Galactooligosaccharide
HACCP	Hazard Analysis Critical Control Point
HADS	Hospital anxiety and depression scale
HAM-D	Hamilton rating scale for depression
HCQ	Health condition questionnaire
HepG2	Human liver hepatocellular cell line
HPA	Hypothalamic-pituitary-adrenal
IBD	Irritable bowel disease
IBS	Irritable bowel syndrome
IDO	Indoleamine-pyrrole 2,3-dioxygenase
IFN-γ	Interferon-gamma
IL	Interleukin
IL-1β	Interleukin-1 beta
LAB	Lactic acid bacteria
LEIDS-r	Leiden index of depression sensitivity-revised
MADRS	Montgomery-Aberg depression rating scale;
MCH	Mean corpuscular hemoglobin
MCHC	Mean corpuscular hemoglobin concentration
MCV	Mean corpuscular volume
MDA	Malondialdehyde
MDD	Major depressive disorder

MIC	Minimum inhibitory concentrations
MMSE-K	Mini-mental status examination-Korean
MRS	de Mann, Rogosa, Sharpe
MTT	3-(4,5)-dimethylthiazol-2-yl-2,5-diphenyltetrazolium bromide
NAMI	National Alliance on Mental Illness
NCBI	National Centre for Biotechnology Information NAMI
NHMS	National Health and Morbidity Survey
NIH	National Institutes of Health
NR	Not reported
PCR	Polymerase chain reaction
PSS-10	Perceived Stress Scale-10
QIDS-SR16	Quick inventory of depressive symptoms
QOL	Quality of life
qPCR	Quantitative polymerase chain reaction
RCT	Randomized clinical trial
RNA	Ribonucleic acid
SCFA	Short-chain fatty acids
SCL90R	Symptom checklist 90-depression
TDO	Tryptophan 2,3-dioxygenase
TH	Tyrosine hydroxylase
TNF-α	Tumour necrosis factor-alpha
TPH-1	Tryptophan hydroxylase-1
TPH-2	Tryptophan hydroxylase-2
TSB	Tryptic Soy Broth
UN	United Nations
WHO	World Health Organization

LIST OF APPENDICES

KESAN *LACTOBACILLUS PLANTARUM* DR7 KE ATAS TEKANAN DAN KEGELISAHAN PADA ORANG DEWASA: KAJIAN RAWAK PLASEBO TERKAWAL, BUTA DWI-PIHAK

ABSTRAK

Kesihatan mental merupakan perkara yang amat rumit. Ianya bukan sahaja menjejaskan kesihatan awam, tetapi juga akan membawa implikasi kepada sosial ekonomi serta kesihatan fizikal secara umumnya. Probiotik telah banyak dilaporkan membawa kebaikan kepada axis-usus-otak. Akhir-akhir ini, terdapat juga banyak minat tertimbul dalam bidang mencari potensi microbiota usus untuk kesihatan mental. Tujuan kajian percubaan rawak, "double-blind" dengan placebo ini adalah untuk menilai ciri-ciri tersebut *Lactobacillus plantarum* DR7 dan mekanisme kemungkinan untuk orang dewasa bawah tekanan selama 12 minggu. Sejumlah 124 orang dewasa yang dibuktikan mempunyai tekanan sederhana dengan menggunakan soal selidik PSS-10 telah dipilih menyertai dalam kajian ini. Mereka dibahagikan scara rawak kepada dua kumpulan samada kumpulan intervensi yang menerima serbuk 1 x 10^9 CFU DR7 ataupun serbuk placebo untuk 12 minggu setiap hari. Sebelum intervensi, data asas telah dikumpul bersama dengan soal selidik kesihatan umum yang dikumpul semasa minggu ke-0, -4, -8 dan -12. Sampel darah dan sample najis pula dikumpul semasa minggu ke-0 dengan -12. Keputusan kajian ini telah mengesahkan DR7 dapat mengurangkan simptom tekanan (P=0.024), gangguan kebimbangan (P=0.001) dan jumlah psikologi skor (P=0.022) seawal-awalnya daripada minggu ke-8 untuk mereka yang mempunyai tekanan berbanding dengan kumpulan plasebo apabila diuji dengan soal selidik DASS-42. Tahap kortisol plasma diperhatikan menurun di kalangan subjek

DR7 berbanding dengan plasebo, berserta dengan penurunan plasma sitokin pro-inflamasi seperti IFN-γ dan TNF-α dan peningkatan plasma sitokin anti-inflamasi seperti IL-10 dan IL-4 ($P < 0.05$). Kumpulan DR7 menunjukkan peningkatan fungsi kognitif dan ingatan yang lebih berkesan seperti perhatian asas, kognisi emosi, dan pembelajaran bersekutu ($P <0.05$) di kalangan orang dewasa (> 30 tahun) berbanding plasebo dan dewasa muda (<30 tahun). Pengambilan DR7 telah meningkatkan kerberkesanannya laluan serotonin seperti yang diperhatikan dengan penurunan ekspresi plasma BH, TH, IDO dan TDO disertai dengan peningkatan ekspresi TPH2 dan 5-HT6 manakala laluan dopamin distabilkan menerusi expresi stabil TH dan DBH selepas 12 minggu berbanding plasebo ($P <0.05$). Kesimpulannya, kajian ini telah mengesahkan bahawa DR7 bukan sahaja memenuhi syarat-syarat sebagai probiotik seperti cadangan FAO/WHO, tetapi juga mempunyai potensi untuk keggunaan phamaseutikal sebagai strategi semulajadi untuk meningkatkan fungsi psikologi, kesihatan kognitif serta ingatan untuk kumpulan dewasa bawah tekanan.

EFFECTS OF *LACTOBACILLUS PLANTARUM* DR7 ON STRESS AND ANXIETY IN ADULTS: A RANDOMISED, DOUBLE-BLIND, PLACEBO-CONTROLLED STUDY

ABSTRACT

Mental health represents a complex and complicated public health problem which has wide-ranging social and economic implications, as well as stark consequences for physical health. Probiotics have been reported to exert beneficial effects along the gut-brain axis. Lately, much interest was generated in the potential role of gut flora in mental disorders. This randomized, double-blind and placebo-controlled human study aimed to evaluate such properties of *Lactobacillus plantarum* DR7 and its possible underlying mechanisms of action in stressed adults for a period of 12 weeks. A total of 124 stressed adults were recruited based on moderate stress levels by using the PSS-10 questionnaire and randomised into two groups to receive either 1 x 10^9 CFU DR7 per sachet powder or placebo powder daily for the total of 12 weeks. Before probiotic intervention, baseline questionnaires were collected while health questionnaires were collected at 0^{th}, 4^{th}, 8^{th} and 12^{th} week. Blood samples and fecal samples were also collected at 0^{th} week and 12^{th} week. Results showed that DR7 reduced symptoms of stress (P=0.024), anxiety (P=0.001), and total psychological scores (P=0.022) as early as 8-weeks among stressed adults compared to the placebo group as assessed by the DASS-42 questionnaire. Plasma cortisol level was reduced among DR7 subjects as compared to the placebo, accompanied by reduced plasma pro-inflammatory cytokines such as IFN-γ and TNF-α and increased plasma anti-inflammatory cytokines such as IL-10 and IL-4 (P<0.05). DR7 can improve cognitive

and memory functions better in normal adults (>30 years old) such as basic attention, emotional cognition, and associate learning ($P<0.05$) as compared to the placebo and young adults (<30 years old). The administration of DR7 enhanced the serotonin pathway, as observed by lowered expressions of plasma DBH, TH, IDO and TDO accompanied by increased expressions of TPH2 and 5-HT6, while stabilizing the dopamine pathway as observed via stabilized expressions of TH and DBH over 12-weeks as compared to the placebo ($P<0.05$). Our results indicated that DR7 fulfil the requirement of a probiotic strain as per recommendation of FAO/WHO and possess potential pharmaceutical application as a natural strategy to improve psychological functions, cognitive health and memory in stressed adults.

CHAPTER 1

INTRODUCTION

1.1 Background

The balance of gut microbiota is playing a key role to our general health and wellbeing. Lately, there is a developing interest about the particular role of gut microbiota towards mood and behaviour regulation, especially on two-ways communications along the microbiome and gut-brain-axis (Clarke et al., 2013, Cryan and O'Mahony, 2011, Mayer, 2011). The axis refers to a chemical signalling between gut and the nervous system. The finding remarks a major breakthrough in science by understanding the interaction of the gastrointestinal (GI) tract with the nervous system. Gut-brain communication also proven to be crucial for human gastrointestinal and psychology health particularly with increased evidence found between the relationship of mental disorder and both inflammatory bowel disorder and the functional bowel diseases (Neufeld et al., 2011, Walker et al., 2008). These unparalleled connections draw the attention of the significant of this system in our homeostasis regulation (Mayer, 2011).

Stress is defined as a nonspecific response of the body towards any threatening demands, causing in anxiety, uneasiness, emotional distress and difficulty in adaptation (George, 2010). It is often triggered by an event and the impacts of stress-induced psychological consequences are gaining increasing interests. Anxiety is the first psychological reaction to stress, where prolonged anxiety often leads to mental illnesses including depression. Globally, more than 300 million individuals are having depression, and this is accompanied by almost 800,000 suicidal deaths yearly (WHO, 2018). The prevalence of mental health problems

among adults in Malaysia also showing an increasing tread. According to the latest National Health and Morbidity Survey 2015, there is an increased from 10.7% from 1996 to 29.2% in 2015. In short, every 3 in 10 adults have mental health problem in Malaysia (NHMS, 2015).

The physiological of stress responses include activation of the Autonomic nervous system (ANS) and the Hypothalamic-pituitary-adrenal (HPA) axis, leading to increased blood and tissue levels of catecholamines and glucocorticoids. These hormones alter immune functions such as antigen presentation, leukocyte trafficking and proliferation, antibody secretion and cytokine release (Dhabharet et al., 1995). Long-term exposure to glucocorticoid leads to increased resistance of the glucocorticoid receptor which ultimately decreases sensitivity of immune cells and impairs downregulation of inflammatory responses, exemplifying the impacts of stress on the immune system (Miller et al., 2002).

A probiotic is defined as “live microorganisms that confer the health effects to the host when consumed in adequate amounts” (FAO/WHO, 2006). While *Lactobacillus* remains as one of the common residents of gut microbiota, they are also the most common bacterial genera reported with probiotic properties, exerting health benefits ranging from regulation of the gut environment, to alleviation of metabolic disorders and modulation of immune responses (Galdeano and Perdigón, 2004).

As there is an increasing affirmation confirmed the interaction of gut microbiota with brain health along the gut-brain axis, a bidirectional flow of signalling responses along the gut and brain (Mayer, 2011). Microbial neuroactive substances and their precursors such as tryptophan have been reported to deliver to the brain through endocrine and afferent autonomic pathways leading to altered

behavioural responses (Desbonnet et al., 2008) and brain development, mood and cognition (Romijn et al., 2008). Germ-free mice showed higher levels of plasma corticosterone accompanied by increased anxiety behaviours as compared to specific pathogen-free mice (Sudo et al., 2004), while the administration of *Lactobacillus rhamnosus* JB-1 reduced anxious and depressive behaviours in mice (Neufeld et al., 2011), illustrating the influence of gut microbiota on brain health and behaviours of the host. In humans, a milk drink containing *Lactobacillus casei* Shirota improved mood scores compared to the placebo group after 3-weeks (Benton et al., 2006), while the administration of *L. casei* Shirota at 2.4 x 10 log CFU/day for 2 months improved anxiety symptoms in patients with chronic fatigue syndrome, a common comorbid of anxiety disorders (Rao et al., 2009). Amid these positive reports, the mechanisms involved remain largely unknown, while issues related to host and strain dependencies remain a primary concern prior to further development of probiotics as an adjuvant or preventive therapy for psychological disorders.

Lactobacillus plantarum DR7 (DR7), a bovine milk isolate, has previously reported have the ability to activate the 5' AMP-activated protein kinase (AMPK) pathway via phosphorylation (Lew et al., 2018), while chronic mild stress has been reported to induce anxiety and depression-like behaviours in mice via the inactivation of AMPK (Zhu et al., 2014). We postulated that DR7 may have the potential to modulate brain health along the gut-brain-axis. This study aimed to investigate the effects of DR7 on stress, anxiety and depression in stressed adults, in addition to memory capacity and cognitive functions. Considering that little information is available on the mode of actions, we also hope to better understand and elucidate the possible mechanisms involved.

1.2 Aim and objectives of research

The aim of this study was to evaluate the potential effects of *Lactobacillus plantarum* DR7 for its brain health benefits through oral consumption for a period of 12 weeks among stressed adults in Malaysia. The specific objectives of this study were:

1) To assess the potential of *Lactobacillus plantarum* DR7 in reducing the stress, anxiety and depression level of stressed adults compared to the placebo group.

2) To determine the effects of probiotic *Lactobacillus plantarum* DR7 on cognitive health and memory improvement of stressed adults compared to the placebo group.

3) To compare the effects of *Lactobacillus plantarum* DR7 on plasma cortisol level and neurotransmitter parameters to the placebo group after 12 weeks.

CHAPTER 2

LITERATURE REVIEW

2.1 Definition of probiotics

Probiotics being lactic acid bacteria (LAB) or *bifidobacteria* offer a series of health benefits. In the early 20th century, LAB such as *lactobacilli* was first reported by Elie Metchnikoff, a Russian scientist, to have beneficial health effects and it was associated to longevity due to the heavy congestion of yogurt (Soccol et al., 2010, Gogineni et al., 2013). Later, Henry Tissier manage to isolate *Bifidobacteria* from the stools of breast-fed baby back in 1899. He suggested to treat infant with diarrhea with the oral intake of *bifidobacteria* (Tissier, 1906). In 1907, the same Russian scientist, Elie Metchnikoff followed his findings then he wrote his famous quote, "The Prolongation of Life". It became the first scientific explanation about the potential of eating substances, may alter the gastrointestinal microflora which enhance human health. After the Japanese microbiologist, Minoru Shirota came across a bacteria that able to survive through the gastrointestinal after oral ingestion, probiotics treatment took a major move forward in 1930. His finding has resulted the first fermented bacteria containing drink that was commercially marketed worldwide today (Gogineni et al., 2013).

The word probiotic is derived from Latin (*pro*) and Greek (*bios*) meaning literally "for life". The German researcher, Ferdinand Vergin proposed the word "probiotika" means that "active substances that are essential for a healthy development of life" back in 1954 (Hamilton et al., 2013). The probiotic definition now was introduced in 2001 by FAO/WHO as "live microorganisms which, when

administered in adequate amount, confer a health benefit to the host". Subsequently, in 2002 FAO/WHO drafted the standard in probiotics evaluation for various of food products.

Probiotics are now commonly applied in different food or health products include functional food and beverages. In the French society back in 1906, fermented milk product which contain *Streptococcus thermophilus* was the first commercialization of probiotics product and follow by yogurt with *Lactobacillus delbreukii* in 1919 (Gogineni et al., 2013). Over recent years, probiotics have gained enormous popularity and great interest (Ringel et al., 2012). Consumers take these probiotic products to expect various beneficial health effects such as improvement of intestinal condition, prevention of diarrhea, alleviation of constipation, stimulation of immunological capacity, and reduction of allergic symptom.

2.2 Probiotics classification

The health benefits of probiotics are strain specific. To identify a potential beneficial bacterium, the strain and phenotypic of the bacteria should be the first approach upon identifying. On top of that, in order to qualify as probiotics that can benefits the host, there are two fundamental selection criteria need to be considered - the strain origin and functional aspects. As of today, various of microorganisms currently being used as probiotics. To classify a bacterium, the first name is the Genus (e.g. *Lactobacillus*). They are the group of bacterium sharing the same quality, for instance physical characteristics, metabolic needs and metabolic end products. The second name of bacterium will be Species (e.g. *plantarum*). Here referring to a much narrower category according to their similar characteristics but differentiate them from one another species. Lastly, the most specific classification is the Strain which separate members of the same species into subgroups according to the bacteria properties that have in common but different within the same species (e.g., strain DR7) (Amirreza et al., 2016).

Being a probiotic, it must a viable bacterial strain and proven to be safe for human consumption. The strain must be tolerance to gastric acidity, resistant to bile acid, good mucin adherence, possess antibacterial activity towards pathogenic microorganisms and able to compete with pathogens on the adherence of mucin surfaces (Fijan, 2014). On top of that, the bacteria must sustain through the GI tract upon consumption and the capable to proliferate in the gut microflora of the host. Other than fulfilling these important properties, probiotics with commercialization values must be also come with a good stability in finished product with high survival rate throughout the product shelf life (Shewale et al., 2014).

2.3 Beneficial effects of probiotics

Both *Lactobacillus* and *Bifidobacterium* are the most general bacteria group which bring health benefits as well as therapeutic effects to the host through modifying host's gut microflora and restore the balance of microbiota (Kailasapathy and Chin, 2000, Rauch and Lynch, 2012). *Lactobacillus* refers to a group of lactic acid producing Gram-positive rods which are obligate and facultative anaerobes in the human GI and genitourinary tracts (Fujisawa et al., 1992, McGoarty, 1993). *Bifidobacterium* is an anaerobic, Gram-positive, nonspore-forming and pleomorphic rod. Bacteria in the *Bifidobacterium* genus produce lactic and acetic acids as by-products through glucose utilization (Amirreza et al., 2016).

Probiotics has been now studied in various GI and non-gastrointestinal disorders and its potential benefit has been proven over the last 2-3 decades. There are several clinical trials as well as the *in-vitro* studies being carried out to investigate the safety of probiotic and the effectiveness in many different diseases such as infectious diarrhea or antibiotic-associated diarrhea and constipation (Gogineni et al., 2013, Hibberd et al., 2015, Shi et al., 2016). The postulated mechanism of action is through the immunomodulation effect by probiotic once they start to colonize in the gut. Also, a great number of probiotics strains have confirmed in clinical study to be able to relive the allergy symptoms with the reduction of plasma immune markers. With that, the efficacy of probiotics in the prevention of allergic rhinitis and others respiratory illness also reported in several studies (Yang et al., 2013). Further on that, probiotics also reported to have the effect on skin and urogenital health (Barrons and Tassone, 2008, Al-Ghazzewi and Tester, 2014, Wang et al., 2016).

The strong correlations of the gut microbiota on human health and homeostasis has many clinical evidences. In many of the recent researches, probiotics also showed potential modulate the pathogenesis of obesity. The possible mechanisms involved including improved microbial balance, decreased food intake, decreased abdominal adiposity and increased mucosal integrity with decreased inflammatory markers (Mallappa et al., 2012). The interest of probiotics for human health has also extended to more in-depth study related to anti-cancer properties and neurodegenerative disorders. In anti-carcinogenic research, studies demonstrated the probiotics regulated gene expressions and signaling pathways for instance cell apoptosis and metastasis as well as DNA oxidative damage prevention. The action of probiotics illustrated through the clinical study is very specific. The *in-vitro* study has showed that probiotics only inhibit the growth of breast cancer cells MCF-7, not the normal mammary epithelial cells (Motevaseli et al., 2017). Lately, with the emerging of gut-brain-axis theory also unveils the usefulness of probiotics in preventing or treating neurological disorder, it is likely through the up-regulating of neurotransmitters such as serotonin, brain- neurotrophic factor (BDNF) and γ-aminobutyric acid (GABA) (Umbrello and Esposito, 2016). Probiotics not only lowering anxiety like behavior, but also promoting tryptophan (serotonergic precursors) which is critical in depression management when tested in both rat and human subjects (Shi et al., 2016). Looking at all the health benefits mentioned herein and the proven efficacy of probiotics, it is inarguable that probiotics will come to the fore as the future natural approach for human health.

2.4 *Lactobacillus* health benefits

2.4.1 General properties of *Lactobacillus*

Lactobacillus are lactic acid bacteria, belonging to the family of *Lactobacillaceae*. Nevertheless, they are one of the most numerous groups of bacteria linked to human with remarkable species that are used for the industrial fermentation of dairy and other food products. *Lactobacillus* are naturally correlated with mucosal surfaces, particularly the gastrointestinal tract, the vagina and the oral cavity (Tannock, 2004).

Currently, 221 *Lactobacillus* species are known (NCBI, 2016). Bacterial strains can further divide more specifically follow their genotype and phenotype. Characteristic from one bacterial strain or speciesnot necessarily the same with another one. LAB represents a remarkably heterogeneous bacterial group which can produce lactic acid. It is either by homofermentative like *L. acidophilus, L. delbrueckii, L. helveticus, L. salivarius* or facultatively heterofermentative including *L. casei, L. curvatus, L. plantarum, L. sakei* and lastly obligately heterofermentative including *L. brevis, L. buchneri, L. fermentum* and *L. reuteri* (Hammes and Vogel, 1995, Ibrahim, 2016, Pot et al., 1994). Within the LAB, the subgroup of the *Lactobacillus* complex has been the key interest because many strains found inhabit ecologic niches in the either humans or animal GI tracts (Klaenhammer and Russell, 2000).

Up to today, there are a numbers of *Lactobacillus* strains are commercialized as probiotics due to their consumption can confer a health benefit to host (Saxelin et al., 2005). They are taken as a friendly bacteria to recolonize particular areas of body in order to give nutritional benefits by enhancing the

mineral absorption (Madsen et al., 1999). *Lactobacillus* also reported can improve the mucosal barrier and decrease intestinal permeability (Shornikova et al., 1997). In the course of antibiotic, *Lactobacillus* probiotics can prevent or minimize the depletion of normal flora and pathogenic bacteria colonization (Alander et al., 1999, Sullivan et al., 2003). In the vagina, lactic acid from *Lactobacillus* able to lower vaginal pH, this can prevent the growth of pathogen (Maggi et al., 2000). While *Clostridium difficile* colonization reported to have association with allergic disease, *Lactobacillus* is postulated to protect children from allergy (Kalliomaki et al., 2001, Sjögren et al., 2009).

All the researchers agreed that *Lactobacillus* and other probiotic strains must be able to colonize an area of tissue in order to have the effectiveness. To achieve this, *Lactobacillus* contained product must be alive and viable organism. For oral consumption, bacteria must also remain viable after passing through the gut, and able to stay on to the intestinal epithelium (Amirreza et al., 2016).

2.4.2 Gastrointestinal microbiota

Gut microbiota is shaped from the moment we are born, through acquisition of mother's commensal bacteria and the exposure to external environment (Hill, 2018, Makino et al., 2013). Microbiota personalization is host specific and the community consistently change throughout different stages of life (Seidel and Valenzano, 2018). These changes are considered part of the natural selection process and is not harmful to the body. A healthy human GI tract is populated with over 500-1000 different species of bacteria, make up several hundred grams of total weight to a human body (Gerritsen et al., 2011, Sjögren et al., 2009). More than 70% of the microorganisms are found in human GI tract,

mainly in the epithelial surface, mucus layer in the intestine or intestine lumen (Picard et al., 2015, Sekirov et al., 2010).

Since 1960s, the possibility of that diet can influence the gut microbiota has been studied in the scientific community. Human diets believed to have direct effects on the microbiome, results in changes of the patterns of biochemical reactions in the intestinal lumen. For instance, nutrients like vitamins, amino acids or soluble fibers that are consumed by the host are assimilated and converted into other metabolites by intestinal microbes (Glendinning and Free, 2014, Hemarajata and Versalovic 2013). The end products of these biochemical conversions like short-chain fatty acids (SCFAs), biogenic amines such as histamine or other amino acids derived metabolites such as serotonin or gamma-aminobutyric acid (GABA) can influence in health and disease states (Rauch and Lynch, 2012, Sartor and Mazmanlan, 2012) and thus may influence microbial composition (Hemarajata and Versalovic 2013).

Dysbiosis can cause disruption between microbes and its host. These changes in microbiome composition and function will lead to the increase of disease susceptibility (Frank et al., 2011). Alterations in the composition of the intestinal microbiome is correlated with infections in the GI tract, inflammatory bowel disease (IBD) and irritable bowel syndrome (IBS) and possibly, some mental disorders, particularly depression, which has been strongly associated with increased immune and inflammatory activation (Berk et al., 2013, Maes et al., 2013). Since homeostasis of the commensal gut microbiota in GI ecosystem is crucial in maintaining host's health (Hawrelak and Myers, 2004, Ohno, 2015). Therefore, treatment modalities to manipulate and restore the balance in the richness and diversity of intestinal microbiome are being explored with the aims of

improving human health (Sonnenburg and Fischbach, 2012).

2.4.3 *Lactobacillus* potential effects on brain health: *in-vivo* evidences

Several probiotic strains were reported to have potential on brain health through animal studies. The administration of *Lactobacillus plantarum* PS128 (PS128) reduced anxiety and depression-like behaviors of mice and significantly decreased inflammation and corticosterone levels. Notably, administration of PS128 significantly increased levels of dopamine and serotonin in the prefrontal cortex and striatum compared with control mice (Liu et al., 2015, Liu et al., 2016). The administration of the single strain *Lactobacillus helveticus* NS8 reduced anxiety, depression and cognitive dysfunction. In addition, *L. helveticus* NS8 increased the serotonin, norepinephrine (NE) and brain-derived neurotrophic factor (BDNF) levels in the hippocampus (Liang et al., 2015). Various preclinical studies summarized in Table 2.1 have demonstrated that administration of probiotics affects emotional behavior in animal models including those using *Lactobacillus* strains (Bravo et al., 2011, Breaed et al., 2012, Desbonnet et al., 2010). Notably, researchers have found a positive role for the human gut microbiota in the gut-brain axis, which can alter minds and behaviors through the CNS through the randomized controlled trials. The effects of probiotics supplementation on depressive symptoms was reported to have a positive effects on depressive symptoms compared to placebo (Miyaoka et al., 2018, Akkasheh et al., 2016, Kazemi et al., 2018, Kouchaki et al., 2017, Majeed et al., 2018, Mohammadi et al., 2016) but some showed no effects (Cremon et al., 2018, Dickerson et al., 2014, Gomi et al., 2018, Roman et al., 2018, Kelly et al., 2017, Romijn et al., 2017, Kato-Kataoka et al., 2016, Ostlund-Lagerstr€om et al., 2016, Simren et al., 2010, Rao et al., 2009, Benton et al., 2007).

Table 2.1 Randomized, placebo-controlled trials of the potential of *Lactobacillus* strains in brain health.

Study Population (age-gender)	Total participants (analysed; total female)	Study Design	Intervention (CFU/day)	Duration	Depression-related Outcomes	Country	References
Patients with IBS (18-65; F/M)	42 (40; 26)	Pilot double-blind placebo-controlled cross-over RCT	*L. paracasei* CNCM I-1572 (2.4×10^{10} CFU)	18 weeks (2w pre-intervention, 4w treatment, 4w washout, 4w treatment, 4w follow-up)	- No significant decrease in HADS depression subscale compared to placebo	Italy	Cremon et al., 2018
Patients hospitalized for mania (18-65; F/M)	83 (66; 42)	Double-blind placebo-controlled RCT	*L. GG* and *B. lactis* Bb12 ($>10^8$CFU)	24 weeks	- No significant improvement in MADRS compared to placebo	USA	Dickerson et al., 2014
Healthy adults with temporary gastric symptom (20-64; F/M)	100 (79; 41)	Double-blind placebo-controlled RCT	100 ml fermented milk ($\geq 3 \times 10^7$CFU/ml of *B. bifidum* YIT 10347 and $\geq 10 \times 10^7$CFU/ml of *S. thermophilus* YIT 2021	6 weeks (2w pre-intervention, 4w intervention)	- No significant effect on Depression-dejection, compared to placebo	Japan	Gomi et al., 2018

Patients with mild to moderate MDD (18-50; F/M)	110 (110; 78)	Double-blind placebo-controlled RCT (3 groups)	Probiotic: *L.helveticus* R0052 and *B. longum* R0175 ($\geq$10 x 10^9 CFU) Prebiotic: 5 g Galactooligosaccharide	8 weeks	- Significant reduction in BDI by probiotic (but not prebiotic) compared to placebo	Iran	Kazemi et al., 2018
Patients with mild to moderate IBS and MDD (20-65; F/M)	40 (40; 34)	Pilot double-blind placebo-controlled RCT	*Bacillus coagulans* MTCC 5856 (2 x 10^9 CFU)	90 days	- Significant improvement in HAM-D, MADRS, CES-D compared to placebo	India	Majeed et al., 2018
Patients with treatment-resistant MDD (NR; F/M)	40 (40; 24)	Open-label RCT with a control group	*Clostridium butyricum* CBM58 (first week: 40 mg 2-8 weeks: 60 mg)	8 weeks	- Significant changes in HAMD-17 and BDI scores, compared to the control group	Japan	Miyaoka et al., 2018
Patients with Fibromyalgia; (NR; F/M)	40 (31; 28)	Pilot double-blind placebo-controlled RCT	ERGYPHILUS Plus (*L. rhamnosus GG*, *L. casei*, *L. acidophilus*, and *B. bifidus*) (12 x 10^6 CFU)	8 weeks	- No significant improvement in BDI scores, compared to placebo	Spain	Roman et al., 2018
Healthy subjects (18-40; M)	NR (29; 0)	Placebo-controlled cross-over RCT	*L. rhamnosus* JB-1 (1 x 10^9CFU)	8 weeks (4w treatment, no washout, 4w switch	- No significant effect of treatment on BDI scores	Ireland	Kelly et al., 2017

Patients with Multiple Sclerosis (18-55; F/M)	60 (60; 50)	Double-blind placebo-controlled RCT	*L. acidophilus, L. casei, B. bifidum* and *L. fermentum* (each 2 x 10^9 CFU)	12 weeks	- Significant reduction in BDI and DASS scores compared to placebo	Iran	Kouchaki et al., 2017
Subjects with low Mood (≥16; F/M)	79 (79; 62)	Double-blind placebo-controlled RCT	*L. helveticus* R0052 and *B. longum* R0175 (3 x 10^9 CFU)	8 weeks	- No significant effect on MADRS, DASS-42 depression subscale, and QIDS-SR16 compared to placebo	New Zealand	Romijn et al., 2017
Patients with MDD (20-55, F/M	40 (40; 34)	Double-blind placebo-controlled RCT	*L. acidophilus, L. casei, B. bifidum* (2 x 10^9 CFU from each)	8 weeks	- Significant reductions in mean BDI score, compared to placebo	Iran	Akkasheh et al., 2016
Medical students to take a stressful examination (22-24; F/M)	51 (47; 21)	Double-blind placebo-controlled RCT	*L. casei Shirota (LCS)* fermented milk containing *L. casei Shirota* (>1.0 x10^{11}CFU)	12 weeks 2w pre-intervention 8w intervention (pre-exam) 2w post-exam	- No significant effects in neither group, and no difference between groups, for HADS-depression score	Japan	Kato-Kataoka et al., 2016

Petrochemical workers (20-60; F/M)	75 (70;34)	Double-blind placebo-controlled RCT (3 groups)	Probiotic Yogurt: at least 1×10^7 CFU *L. acidophilus* LA5 and *B. lactis* BB12 Capsule: *L. casei* (3×10^3), *L. acidophilus* (3×10^7), *L. rhamnosus* (7×10^9), *L. bulgaricus* (5×10^8), *B. breve* (2×10^{10}), *B. longum* (1×10^9), *S. thermophilus* (3×10^8) CFU/g	6 weeks	- Improved DASS and GHQ scores by both probiotic capsule and probiotic yogurt, but no significant effect compared to placebo	Iran	Mohammadi et al.,2016
Free-living older adults (≥65; F/M)	290 (249;128)	Double-blind placebo controlled RCT	*L.reuteri* DSM 17938 (1×10^8 CFU)	12 weeks	- No significant effect on HADS depression score compared to placebo, neither in the whole sample nor GI disorders participants	Sweden	Ostlund-Lagerstr€om et al., 2016

Healthy adults (NR; F/M)	40 (40; 32)	Triple-blind placebo-controlled RCT	2 g Powder of Ecologic-Barrier (*B. bifidum* W23, *B. lactis* W52, *L. acidophilus* W37, *L. brevis* W63, *L. casei* W56, *L. salivarius* W24, *L. lactis* (W19 and W58) (2.5×10^9 CFU/g)	4 weeks	- No significant reductions in BDI compared to placebo, but significantly improved total LEIDS-r	The Netherlands	Steenbergen et al., 2015
Healthy adults (30-60; F/M)	66 (55; 41)	Double-blind placebo-controlled RCT	*L. helveticus* R0052 and *B. longum* R0175 (3×10^9 CFU)	30 days	- No significant decrease in HADS-depression compared to placebo, at follow-up	France	Messaoudi et al., 2011
Patients with IBS (18-70; F/M)	74 (74; 52)	Double-blind placebo-controlled RCT	200 ml (twice daily) milk fermented with the yoghurt bacteria *L. bulgaricus* and *S. thermophiles,* and *L. paracasei, ssp. paracasei* F19,	8 weeks	- No significant decrease in HADS-depression compared to placebo	Sweden	Simren et al., 2010

			L. acidophilus La5, *B. lactis* Bb12 (at least 5 x 10^7 CFU/mL)				
Chronic fatigue syndrome (CFS) (18-65, F/M)	39 (35; 27)	Pilot double-blind placebo-controlled RCT	*L. casei Shirota* (LCS) (24 x 10^9 CFU)	8 weeks	- No significant reduction in BDI scores, compared to baseline or placebo	Canada	Rao et al., 2009
Healthy adults (48-79, F/M)	132 (126; 75)	Double-blind placebo-controlled RCT	65 ml milk drink containing *L. casei* Shirota (at least 6.5 x 10^9 CFU)	20 days	- No significant improvement in depressed/elated dimension compared to placebo (except among those who were in the lowest tertile of mean daily ratings or baseline POMS depression)	UK	Benton et al., 2007

BDI= Beck depression inventory; CES-D= Center for epidemiological studies depression scale; DASS= Depression, anxiety and stress scale; GDS-SF= Geriatric depression scale-short form; GHQ-28= 28-item General health questionnaire; HADS= Hospital anxiety and depression scale; HAM-D= Hamilton rating scale for depression; IBS= Irritable bowel syndrome; LEIDS-r= Leiden index of depression sensitivity-revised; MADRS= Montgomery-Aberg depression rating scale; MDD= Major depressive disorder; MMSE-K= Mini-mental status examination-Korean; NR= Not reported; QIDS-SR16= Quick inventory of depressive symptoms; RCT= Randomized clinical trial; SCL90R-Dep= Symptom checklist 90-depression.

2.5 *Lactobacillus plantarum* DR7 properties

Lactobacillus plantarum DR7 showed the ability to adhere to mucin (Figure 2.1a) and tolerated with simulated conditions of gastric acidity and bile (Figures 2.1b, c). DR7 showed better resistance towards acid while maintaining viability (reduction of less than one log CFU) as compared to bile conditions, where 50% of viability was maintained. Cell free supernatant of DR7 showed antioxidant potential and surpassing that of the standard antioxidant Trolox (Figure 2.1d). DR7 also able to adhere to non-antibiotic resistance as per requirement of EFSA (Table 2.2). Analytic Profile Index (API) assessment revealed that DR7 was able to utilize sugars such as L-arabinose, D-ribose, D-galactose, D-glucose, D-fructose, D-mannose, D-mannitol, D-sorbitol, methyl-α-D-mannopytanoside, N-acetylglucosamine, amygdalin, arbutin, esculin ferric citrate, salicin, D-cellobiose, D-maltose, D-lactose, D-melibiose, D-saccharose, D-trehalose, D-melezitose, D-raffinose, gentiobiose, D-turanose and potassium gluconate (Table 2.3). DR7 could utilize shorter chained galactose-based oligosaccharide prebiotic such as GOS better than fructose-based oligosaccharide prebiotic such as FOS and could not thrive well in longer-chained oligosaccharide prebiotic such as inulin (Figure 2.2). Antimicrobial assays showed that cell free supernatant of DR7 exhibited inhibitory activities against common pathogens such as *S. aureus*, *S. epidermidis* and *E. coli*, where DR7 outperformed the antibiotics used against *S. epidermidis* and *E. coli*. (Figure 2.3).

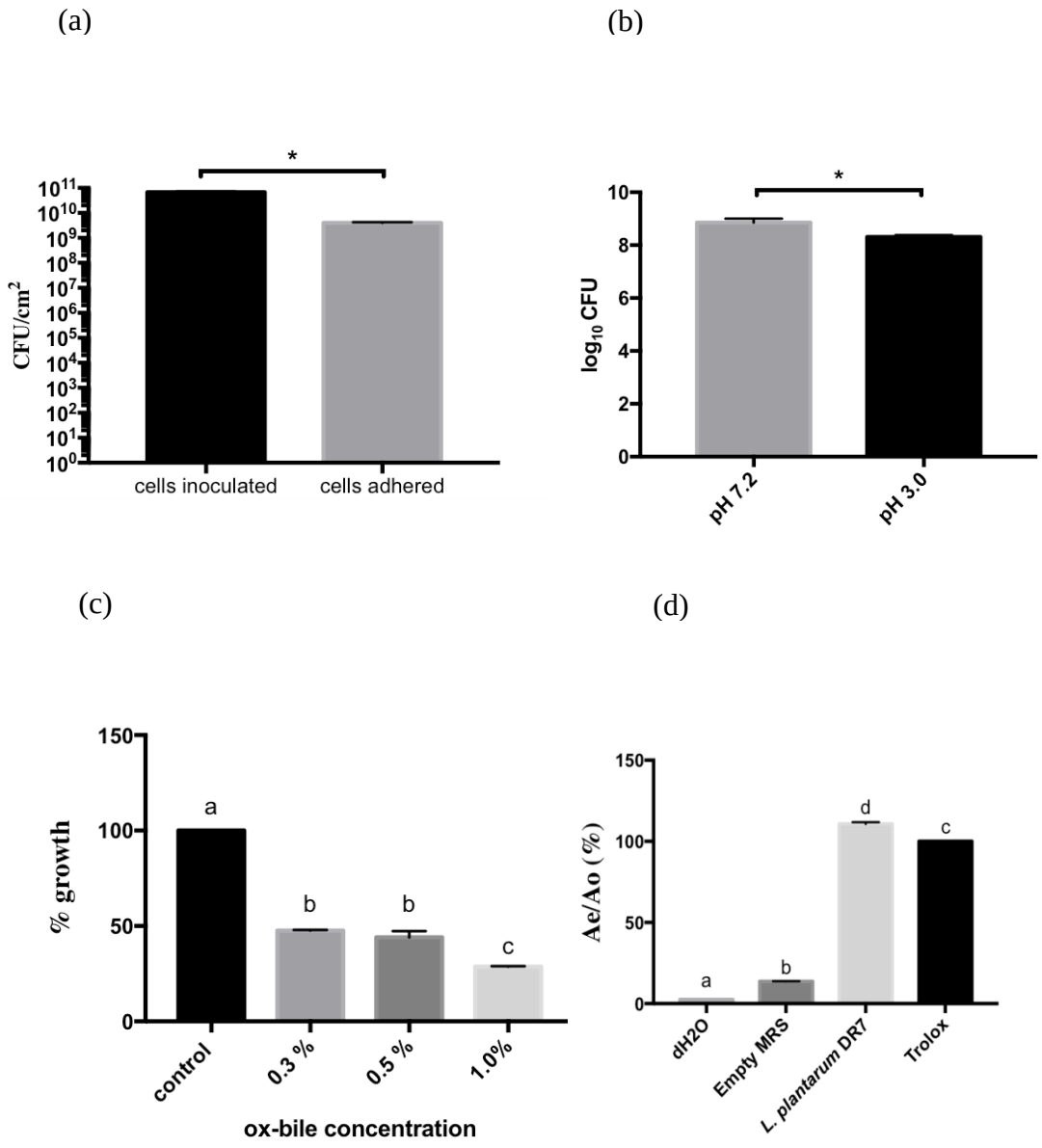

Figure 2.1 Probiotic properties of *Lactobacillus plantarum* DR7. (a) Adhesion to mucin (CFU/cm^2) of loaded and adhered cells onto mucin in 96-well microplate. (b) Resistance to acid (viability $\log_{10}$ CFU) at pH 7.0 and pH 3.0 phosphate buffered saline after incubation at 37 °C for 3 h. (c) Resistance to bile (percent growth as compared to control) at different concentrations of ox-bile after incubation at 37 °C for 24 h. MRS broth was used as the base medium. (d) Ferric reducing antioxidant power of cell-free supernatant. Trolox was used as positive control, while distilled water (dH_2O) and empty MRS was used as negative control. All data are expressed as mean and error bars represent standard errors of means (n=6). * Significantly different as compared to the control via independent T-test ($P<0.01$). Different letters indicate statistical difference ($P<0.05$) as determined by one-way ANOVA.

Table 2.2 Minimum inhibitory concentration (mg/l) for *Lactobacillus plantarum* DR7 tested against commercial antibiotics according to the guidelines by European Food Safety Authority (EFSA) using broth microdilution method. MIC value was recorded as the lowest concentration of antibiotic that prevented visible bacterial growth.

Antibiotics	Microbiological cut-off value (mg/l) for *L. plantarum*	
	EFSA	DR7
Gentamicin	16	0.5
Kanamycin	64	8
Tetracycline	32	16
Erythromycin	1	0.25
Clindamycin	2	1
Chloramphenicol	8	8
Ampicillin	2	1

Table 2.3 Carbohydrate utilization of *Lactobacillus plantarum* DR7 as measured using API-50 CHL with 24 h incubation at 37 °C.

Active ingredients	Reaction†	Active ingredients	Reaction†
control	_	Arbutin	+
Glycerol	_	Esculin ferric citrate	+
Erythritol	_	Salicin	+
D-arabinose	_	D-cellobiose	+
L-arabinose	+	D-maltose	+
D-ribose	+	D-lactose (bovine origin)	+
D-xylose	_	D-melibiose	+
L-xylose	_	D-saccharose	+
D-adonitol	_	D-trehalose	+
Methyl-β-D-xylopyranoside	_	lnulin	_
D-galactose	+	D-melezitose	+
D-glucose	+	D-raffinose	+
D-fructose	+	Amidon (starch)	_
D-mannose	+	Glycogen	_
L-sorbose	_	Xylitol	_
L-rhamnose	_	Gentiobiose	+
Dulcitol	_	D-turanose	+
Inositol	_	D-lyxose	_
D-mannitol	+	D-tagatose	_
D-sorbitol	+	D-fucose	_

Methyl-α-D-mannopytanoside	+	L-fucose	_
Methyl-α-D-glucopyranoside	_	D-arabitol	_
N-acetylglucosamine	+	L-arabitol	_
Amygdalin	+	Potassium gluconate	+
		Potassium 2-ketogluconate	_
		Potassium 5-ketogluconate	_

† + indicates positive reaction; – indicates negative reaction.