[Frontiers In Bioscience, Elite, 10, 344-351, March 1, 2018]

Modifying progression of aging and reducing the risk of neurodegenerative diseases by probiotics and synbiotics

Huey Shi Lye[1], Yee Teng Lee[2], Shao Yin Ooi[1], Lai Kuan Teh[3], Lay Ngor Lim[3], Loo Keat Wei[2]

[1]Department of Agricultural and Food Science, Faculty of Science, Universiti Tunku Abdul Rahman, Jalan Universiti, Bandar Barat, 31900, Kampar, Perak, Malaysia, [2]Department of Biological Science, Faculty of Science, Universiti Tunku Abdul Rahman, Jalan Universiti, Bandar Barat, 31900, Kampar, Perak, Malaysia, [3]Department of Biomedical Science, Faculty of Science, Universiti Tunku Abdul Rahman, Jalan Universiti, Bandar Barat, 31900, Kampar, Perak, Malaysia

TABLE OF CONTENTS

1. Abstract
2. Introduction
3. Aging in relation to neurodegenerative diseases
4. Microbiome, one carbon metabolism and aging
5. Neurodegenerative diseases and synbiotic effect
    5.1. Probiotics can change the manifestation of diseases
    5.2. Synbiotic
6. Summary and prospective
7. Acknowledgement
8. References

1. ABSTRACT

Aging, which affects most of the multi-cellular organisms, is due to a potentially complex set of mechanisms that collectively cause a time-dependent decline of physiological functions. Aging restrains longevity and leads to neurodegenerative diseases including dementia, Alzheimer's disease and lacunar stroke. Human microbiota is now considered to have a strong impact on the progression of aging. The impact of aging and the risk of neurodegenerative diseases can be reduced by using probiotics, or preferably by combining probiotics and prebiotics, also known as synbiotics, that can drastically modify the composition of gut microbiome.

2. INTRODUCTION

In 2014, 7.7% of the population within East Asian countries such as China, Hong Kong, Japan, North Korea and South Korea were older than 65 years. However, the percentage of aging population in these East Asian countries might spike to 14.5% by the year of 2025 and 15% of the total population in Malaysia will be 60 years old or older by the year of 2030 (1-2).

Aging is a time dependent ongoing deterioration of the physiological functions at the level of cells and tissues and at the organismal level (3-5). The progression of aging is due to the existence of potentially complex mechanisms that limit the lifespan of an organism pro-actively beyond its specific age. The lifespan of an organism is affected by extrinsic factors, including extreme population density and unfavorable environmental factors such as scarcity of food and drought condition (6). Nine hallmarks of aging are found at the cellular level, which include genomic instability, telomere attrition, epigenetic alterations, loss of proteostasis, deregulated nutrient sensing, mitochondrial dysfunction, cellular senescence, stem cell exhaustion and altered intercellular communication (4-6). Among these, the genome instability, telomere attrition, epigenetic alteration and loss of proteostasis are the primary hallmarks of aging which trigger the initiation and progression of the aging process. Being an antagonistic hallmark, an optimum deregulated nutrient sensing may help in survival, whereas, an excessive deregulation of nutrient sensing may lead to pathological changes, thus promoting aging. Integrative hallmarks of aging such as stem cell exhaustion and altered intercellular communication tend to act on tissue homeostatic mechanisms to compensate aging-associated damages at cellular level (5).

Aging also leads to the formation of senescent cells which express a senescence-associated secretory phenotype (SASP) and leads to the release

of inflammatory factors (i.e. cytokines, chemokines, growth factors and stromelysin) that suppress somatic and stem cell proliferation (7, 8). SASP is associated with aging-related cognitive impairment arterial stiffness and neurodegenerative diseases such as Alzheimer's disease, dementia and lacunar stroke (7, 9).

3. AGING IN RELATION TO NEURODEGENERATIVE DISEASES

Neurodegenerative diseases are chronic and progressive disorders that usually occur in later stage of life. They are commonly characterized by a gradual loss of neurons and synaptic connections, while displaying variable symptoms, often leading to death (10). The most prevalent example of neurodegenerative disease is dementia. Dementia is a syndrome associated with loss of intellectual abilities such as memory, thinking and orientation, comprehension, learning capacity as well as language and judgment. These conditions are severe enough to influence the daily activities. It is also one of the major causes of dependency and disability among the aging population (11). According to World Health Organization fact sheet in 2016, approximately 47.5 million of individuals worldwide are suffered from dementia, and about 7.7 million of new cases are reported annually.

About 60% - 70% of all dementia are attributable to Alzheimer's disease, while the remaining cases are due to vascular dementia. Manifestations of syndromes associated with dementia are related to damages at specific regions of the brain. For example, aged patients with dementia who suffer from brain atrophy may experience memory loss with poor cognitive skill (12, 13). According to the Alzheimer's association, the initial symptom of Alzheimer's disease is a gradual loss of the ability in remembering new information (14). This early symptom of memory loss is due to damage of brain cells in the hippocampus region, which is a center of learning and memory. Subsequently, patients with Alzheimer's disease may also experience difficulty in solving problem and conducting routine tasks (15).

The risk of developing Alzheimer's disease doubles every 5 years after the age of 65 and symptoms can manifest in elderly who are in their 80s or 90s. (16). Alzheimer's disease is known to be insidious, with gradual worsening of symptoms over a number of years. Patients with advanced Alzheimer's disease show shrinkage in the brain due to cell death, leading to the loss of synapse flow (14). Lacunar stroke which accounts for about one-fourth of all brain infractions is due to blockage of the blood vessels in the basal ganglia, deep cerebral white matter, thalamus and internal capsule (17-18). Patients at age 60 or older who suffer from a large infarct develop silent lacunar infarction (19-20). Cai *et al* suggested that the prevalence of lacunar infarct increases before the age of 60 while its prevalence decreases after age 69 years (21). A study suggests a close relationship between the size of brain infarction and the severity of Alzheimer's disease with patients who suffer from such lacunar infarctions are 4 to 12 times more risk of developing dementia (22, 23).

4. MICROBIOME, ONE CARBON METABOLISM AND NEURODEGENERATIVE DISEASES

Human microbiome refers to the microbiota including bacteria, archaea, viruses and eukaryotes that colonize different parts of the human body. Eight main phyla of bacteria, including Actinobacteria, Bacteroidetes, Fusobacteria, Firmicutes, Lentisphaerae, Proteobacteria, Spirochaetes and Verrucomicrobia reside predominantly in oral mucosa, mammary gland, lungs, respiratory tract, skin surface and gastrointestinal tract. The richness and uniformity of bacterial cell densities are increased from the oral cavity to the anus (24). Human gastrointestinal tract contains about 10 times the number of microbiota that live elsewhere in the body (25). Due to differential selective pressures on the microbiota, 75% of microbiome in the gut is comprised of *Firmicutes* and *Bacteroidetes*. The predominant genera in the colon are *Bacteroides, Porphyromonas, Bifidobacterium (B.), Lactobacillus (L.)* and *Clostridium (C.)*. The enzymes, bile, gastric and pancreatic secretions modify the composition of the gut microbiota throughout the gastrointestinal tract. In addition, the microbial compositions in the intestinal mucosal layer are different than those that reside in the intestinal lumen. Since mucosa-associated bacteria such as *Bacteroides*, *Bifidobacterium*, *Streptococcus*, *Enterococcus*, *Clostridium*, *Lactobacillus* and *Ruminococcus* are found to have close contact with the host cells, they are able to interact with the host immune system and regulate the host immune response that has been implicated in the neurodegenerative diseases (25, 26). In fact, the host immune response has always been involved in acute lacunar stroke, whereby, the innate immune response is augmenting an intravascular inflammatory cascade in the brain. With the initiation of innate immune response after cerebral ischemia, the host immune system has been linked to the post-stroke immunosuppression.

The non-pathogenic *Escherichia (E.) coli* strains such as *E. coli* K-12 can cross the mucosa, invading the bloodstream and causing fatal systemic infection (27, 28). Gram-negative bacteria of phylum *Proteobacteria* such as *E. coli,* and phylum *Verrucomicrobia* such as *Akkermansia muciniphila*, are reported to adhere and reside within the mucus layer and use mucus as the carbon and nitrogen sources. On the contrary, bacterial species of phylum *Firmicutes* such as *Clostridium*, *Lactobacillus* and *Enterococcus*

that predominate in the intestinal lumen are found to be more relevant for the metabolic interactions of food or the products of digestion (25, 26). Different choices of carbon and nitrogen sources are due to the fact that simple carbohydrates from food are being digested and absorbed throughout the digestive system, leaving the indigestible complex polysaccharide in the gut. This complex polysaccharide can be digested by facultative anaerobes *Lactobacillus* and *Enterococcus*. Complex polysaccharide fermentations in the colon are helping those facultative anaerobes to obtain carbon sources for their sustainability. The capability of fermenting polysaccharide shows that these bacteria are able to interact directly with dietary antigens, as they are involved in the digestion of partially digested food and assist in the absorption of nutrients (29).

A number of gut microbiota such as genera *Clostridium* and *Lactobacillus* are involved in the biosynthesis of water-soluble vitamin B. The components of vitamin B such as folic acid (vitamin B_9), pyridoxine (vitamin B_6) and cobalamin (vitamin B_{12}) provide coenzymes which participate in the one carbon metabolism, a series of essential biochemical reactions that starts with the transfer of one carbon atom from serine or glycine to tetrahydrofolate to form 5,10-methylenetetrahydrofolate. This latter reaction is catalyzed by vitamin B_6. The 5,10-methylenetetrahydrofolate is then reduced to a circulating form of folate, 5-methyltetrahydrofolate which can convert homocysteine to methionine, regenerating tetrahydrofolate using vitamin B_{12} as a cofactor (30). Being a pseudo-amino acid, homocysteine is also produced from methionine synthesis pathway with the help of vitamins B_6, B_9 and B_{12}. Homocysteine is the agonist to metabotropic (groups I and II) glutamate receptors. Homocysteine acts as the ionotropic glutamate receptors for amino-3-hydroxy-5-methyl-4-isoxazolepropionate and N-methyl-D-aspartate. Severe dietary deficiencies in the aforementioned vitamins lead to the accumulation of homocysteine and hyperhomocysteinemia. Hyperhomocysteinemia increases calcium influx for excitotoxicity event that subsequently induces oxidative injury in the nerve terminals by promoting free radical production. As an ionotrophic receptor, homocysteine stimulates N-methyl-D-aspartate to enhance adverse oxidative stress. For these reasons, elevated levels of homocysteine in plasma and/or serum have been strongly linked to neurodegenerative diseases (31). Homocysteine is also strongly associated with global DNA methylation levels. A high level of homocysteine can cause a high production of S-adenosyl-homocysteine (SAH), which results in global DNA hypomethylation, especially within the brain tissue (32, 33). Global DNA hypomethylation can suppress homocysteine-induced *cyclin A* gene transcription, inhibiting the growth of endothelial cells. An impaired regeneration of endothelial cells can also cause atherosclerosis and increase the chance of lacunar stroke. Both *Lactobacilli* such as *L. plantarum* as well as bifidobacteria contribute to the folate synthesis. *B. bifidum* contributes to a high level of folate synthesis; while *B. breve* only produces folate at a lower level (34). An insufficient number of *B. bifidum* can jeopardize one carbon metabolism, and directly or indirectly affect nucleotide (purines and thymidine) biosynthesis, redox defense and amino acid homeostasis. In fact, a reduction of *B. bifidum* has direct consequences on homocysteine homeostasis (28). Thus, the lowering of homocysteine level by the administration of probiotics or synbiotics may help in reducing the risk of lacunar stroke (31).

Some studies indicate that homocysteine can disrupt blood-brain barrier (33, 35, 36). Homocysteine causes an imbalance in matrix metalloproteinase 9 (MMP-9) and tissue inhibitor of metalloproteinase-4 (TIMP-4) productions. An elevated level of MMP-9 compromises blood-brain barrier by acting on extracellular matrix and junction proteins (33, 35). It has been suggested that certain gut microbiota can modulate the blood-brain barrier by decreasing its permeability and increasing the expression of the tight junction proteins (37). On the other hand, gut microbiota such as *C. tyrobutyricum* and *Bacteroides thetaiotaomicron* are capable of producing short-chain fatty acids which can strengthen the structure of blood-brain barrier by increasing the protein expression at the tight junction (38).

The enteric nervous system communicates with the central nervous system through neuroendocrine and metabolic pathways. The gut-brain axis provides a potential pathway for the intestinal microbiota and their metabolites to access the brain, integrating the neural, hormonal and immunological signaling between the gut and the brain (26). Enteric microbiota has a great impact on the gut-brain-axis, by interacting with the intestinal cells, central nervous system and enteric nervous system (39). The most direct path that connects the brain and intestine is via the vagus nerve, providing a pipeline for the materials to pass from the intestine to the brain. The changes in the composition of gut microbiota are tightly linked with several neurodegenerative diseases such as dementia, Alzheimer's disease and lacunar stroke. Risk factors such as aging and obesity are influenced by the changes of gut microbiota where a lower ratio of *Bacteroidetes/Firmicutes* increases the risk of lacunar stroke; while a higher *Bacteroidetes/Firmicutes* ratio promotes the up-regulation of proteins in the tight endothelial junction that protects blood-brain barrier from being breached (40).

The gut microbiota is also involved in preventing the overgrowth of pathogens by stimulating the defense mechanism in the gastrointestinal tract.

For instance, lactobacilli can weaken the binding of pathogenic *E. coli* and *Salmonella enteritidis* to the ileal mucosa. Increasing evidences have shown the protective role of probiotics against gastrointestinal pathogens by modulating human gut microbiota. An increased level of *Bifidobacterium* and diversified species were found in elderly individuals who consumed probiotics (10^9 cfu/ml) *B. longum* 46 and *B. longum* 2C for 6 months (41-42). Rampelli and coworkers found that the consumption of probiotics *B. longum* Bar33 and *L. helveticus* Bar13 (10^9 CFU) for one month could modulate the intestinal microbiota by decreasing the opportunistic pathogens *C. cluster* XI, *C. difficile*, *C. perfringens*, *Enterococcus faecium* and the enteropathogenic genus *Campylobacter* (42). Everard and coworkers reported that the treatment with probiotic *Saccharomyces boulardii* dramatically changed the gut microbiota composition of leptin-resistant obese and type 2 diabetic mice (db/db). Daily oral administration of *Saccharomyces boulardii* (120 mg) for 4 weeks increased the proportion of *Bacteroidetes* and decreased the phyla *Firmicutes*, *Proteobacteria* and *Tenericutes* in the gut of db/db mice, suggesting an inversed correlation between *Bacteroidetes* and fat mass (43).

5. NEURODEGERATIVE DISEASES AND SYNBIOTIC EFFECT

5.1. Probiotics can change the manifestation of diseases

Probiotics have been used and studied in various diseases including dementia, Alzheimer's disease and lacunar stroke. A randomized, double-blind and controlled trial was conducted by Akbari and coworkers to study the effects of probiotic supplementation on cognitive function and metabolic status in 60 patients with Alzheimer's disease. The patients were randomly divided into two groups, and were treated with either milk (control) or probiotic milk containing *L. acidophilus*, *L. casei*, *B. bifidum* and *L. fermentum* (2×10^9 CFU/g each) for 12 weeks. Results showed that probiotics mixture significantly ($p<0.05$) improved the mini-mental state examination score ($p<0.001$), malondialdehyde ($p<0.001$), high sensitivity C-reactive protein ($p<0.001$), markers of insulin metabolism ($p = 0.006$) and triglycerides levels ($p = 0.003$) of the Alzheimer's disease patients, which indicating the positive effect of probiotics on cognitive function and individuals' metabolic statuses (44).

Since dementia and Alzheimer's disease can result from lacunar stroke, it is hypothesized that interventions that reduce the risk of lacunar stroke might lower the occurrence of dementia or Alzheimer's disease. Other than diabetes, hypertension is strongly associated with lacunar stroke and the latter is the main risk factor for subcortical ischemic vascular disease (45). Thus, treatment of hypertension might be an alternative way to reduce dementia or Alzheimer's disease. Probiotics have been shown to reduce the risk of hypertension by improving the metabolism. A systemic review and meta-analysis of randomized, controlled trials found that consumption of probiotics significantly reduces ($p<0.05$) systolic and diastolic blood pressures of study subjects, suggesting that probiotics might be used as a potential therapeutic strategy to reduce hypertension (46). Another meta-analysis done by Dong and coworkers had systematically examined the effect of probiotic fermented milk on blood pressure. A significant reduction ($p<0.05$) was observed in the systolic and diastolic blood pressures of pre-hypertensive and hypertensive subjects as compared to placebo (47). Moreover, a probiotic fermented milk also showed a slightly greater effect on systolic blood pressure in hypertensive participants than that of normotensive subjects. Seppo and coworkers conducted a randomized placebo-controlled study involving 39 hypertensive patients treated with 150 mL/d of either test or control product for 21 weeks after 2 weeks run-in period. Administration of milk fermented by *L. helveticus* LBK-16H showed a decreased in systolic ($p<0.05$) blood pressure. It was proposed that *L. helveticus* strains possess a high proteolytic activity and produce bioactive peptides that inhibit the angiotensin converting enzyme activity, which contributes to the antihypertensive effect (48). The antihypertensive effect of *L. helveticus* was also proven in another study done by Aihara and coworkers (49). Daily administration of the supplementary tablets containing powdered *L. helveticus*-fermented milk successfully reduced the elevated blood pressure in subjects with mild hypertension without any adverse effects. A similar observation was also reported by Gómez-Guzmán and coworkers, where a reduced blood pressure was found in spontaneously hypertensive rats (SHR) upon the administration of *L. fermentum* CECT5716 (LC40) or a mixture of *L. coryniformis* CECT5711 and *L. gasseri* CECT5714 (K8/LC9; 1:1) at a dose of $3.3. \times 10^{10}$ CFU/day for 5 weeks (50). These experimental findings suggest the usage of probiotics in controlling blood pressure and reducing the risk of lacunar stroke, which might subsequently reducing the risk of dementia and Alzheimer's disease.

5.2. Synbiotic

Prebiotics are non-digestible food ingredients which selectively stimulate the growth and/or activity of one or a limited number of bacterial species, who have already resided in the colon (51). Non-digestible carbohydrates such as galactooligosaccharides, fructooligosaccharides, lactulose, lactosucrose, polydextose and inulin are commonly used as prebiotics (52).

Combination of probiotics and prebiotics, also known as synbiotics, has shown beneficial synergistic effects in reducing the risks of dementia and

Alzheimer's disease. Using an *in vivo* study, Su and coworkers found that fructooligosaccharides and inulin significantly (p<0.05) enhanced survival and prolonged the retention period of *L. acidophilus* LAFTI L10 (L10), *B. lactis* LAFTI B94 (B94) and *L. casei* L26 LAFTI (L26) (53). Another study by Adebola and coworkers found that prebiotics, lactulose and lactobionic acid increased the tolerance of *L. acidophilus* NCFM and *L. reuteri* NCIMB 11951 to cholic and taurocholic acid significantly (p<0.05). The authors suggested that prebiotics might act as an alternative energy source for lactobacilli which allow them to react better to the bile acid stress (54). Another study by Gopal and coworkers (55) showed that dietary consumption of milk with prebiotic derived from oligosaccharides and a probiotic bacterium *B. lactis* HN019 increased the proportion of bifidobacteria and lactobacilli in the human gastrointestinal tract. Piirainen and coworkers used a randomized, double-blind and crossover study involving 30 healthy children (16 boys, 14 girls) and compared the effects of synbiotic and probiotic alone on the intestinal microbiota. After a 3-weeks study period, a daily consumption of 65 ml of synbiotic milk based fruit juice containing *L. rhamnosus* GG (LGG) (6.5. x 10^9 CFU) and galactooligosaccharides (2g) showed a significantly increased (p<0.001) number of bifidobacteria as compared to those who consumed the probiotic LGG (6.5. x 10^9 CFU) alone (56). Synbiotic therapy was more effective in treating certain diseases than therapies that were limited to probiotics or prebiotics individually (57, 58). These results show that synbiotics appear to be more advantageous than using probiotic or prebiotic alone. However, more *in vitro* and *in vivo* assessment are needed before commercialization of such products can be offered for therapeutic uses.

6. SUMMARY AND PERSPECTIVE

Gut microbiota plays an essential role in neurodegenerative diseases, particularly on dementia, Alzheimer's disease and lacunar stroke. Probiotics and synbiotics appear to have positive impacts on human microbiome via gut-brain axis by reducing homocysteine level, strengthening intestinal barrier, suppressing growth of pathogens and these impacts can decrease the risk of neurodegenerative diseases.

7. ACKNOWLEDGEMENT

We acknowledge the financial support from a Fundamental Research Grant Scheme (FRGS/1/2015/SKK08/UTAR/02/3).

8. REFERENCES

1. A Walker, C Aspalter: The concept of active aging. In active ageing in Asia. *Routledge*, 14-30 (2014)

2. SA Samad, N Mansor: Population ageing and social protection in Malaysia. *Malays J Econ Studies* 50, 139-156 (2013)

3. D Harman: The aging process. *Proc Natl Acad Sci USA* 78, 7124-7128 (1981) DOI: 10.1073/pnas.78.11.7124

4. M Davidovic, G Sevo, P Svorcan, DP Milosevic, N Despotovic, P Erceg: Old age as a privilege of the "selfish ones". *Aging Dis* 1, 139-146 (2014)

5. C López-Otín, MA Blasco, L Partridge, M Serrano, G Kroemer: The hallmarks of aging. *Cell* 153, 1194-1217 (2013) DOI: 10.1016/j.cell.2013.05.039

6. TC Goldsmith: On the programmed/non-programmed aging controversy. *Biochemistry Mosc* 77, 729-732 (2012) DOI: 10.1134/S000629791207005X

7. J Campisi, L Robert: Cell senescence, role in aging and age-related diseases. *Interdiscip Top Gerontol* 39, 45-61 (2014) DOI: 10.1159/000358899

8. A Freund, AV Orjalo, PY Desprez, J Campisi: Inflammatory networks during cellular senescence: causes and consequences. *Trends Mol Med* 16, 238-246 (2010) DOI: 10.1016/j.molmed.2010.03.003

9. A Brunet, SL Berger: Epigenetics of aging and aging-related disease. *J Gerontol A Biol Sci Med Sci* 69, 17-20 (2014) DOI: 10.1093/gerona/glu042

10. MF Mendez, AM McMurtray. Neurodegenerative disorders. In: Encyclopedia of Stress (Second edition). Eds: G Fink, B McEwen, ER de Kloet, R Rubin, G Chrousos, A Steptoe, N Rose, I Craig, G Feuerstein, *Elsevier*, Netherlands (2007) DOI: 10.1016/B978-012373947-6.00575-4

11. World Health Organization (WHO). (2016). Dementia. Available at: http://www.who.int/mediacentre/factsheets/fs362/en/

12. T Niccoli, L Partridge: Ageing as a risk factor for disease. *Curr Biol* 22, 741-752 (2012) DOI: 10.1016/j.cub.2012.07.024

13. G Tosto, TD Bird, DA Bennett: National institute on aging late-onset alzheimer disease/national cell repository for alzheimer disease (NIA-LOAD/NCRAD) Family Study Group. The role of cardiovascular risk factors

and stroke in familial alzheimer disease. *JAMA Neurol* 73, 1231-1237 (2016)
DOI: 10.1001/jamaneurol.2016.2539

14. Alzheimer's Association. Alzheimer's disease facts and figures. *Alzheimer's & Dementia* 10, 47-92 (2014)
DOI: 10.1016/j.jalz.2014.02.001

15. SS Adav, SK Sze: Insight of brain degenerative protein modifications in the pathology of neurodegeneration and dementia by proteomic profiling. *Mol Brain* 9, 1-22 (2016)
DOI: 10.1186/s13041-016-0272-9

16. National Institutes of Health (NIH). Preventing Alzheimer's disease: what do we know? *National Institute on Aging*, 1-24 (2012)

17. A Arboix, L Blanco-Rojas: Cognitive impairment in ischemic lacunar stroke. *Eur Neurol Rev* 8, 144-148 (2013)
DOI: 10.17925/ENR.2013.08.02.144

18. JM Wardlaw: What causes lacunar stroke? *J Neurol Neurosurg Psychiatry* 76, 617-619 (2005)
DOI: 10.1136/jnnp.2004.039982

19. M Hollander, ML Bots, AI Del Sol, PJ Koudstaal, JCM Witteman, DE Grobbee, A Hofman, MMB Breteler: Carotid plaques increase the risk of stroke and subtypes of cerebral infarction in asymptomatic elderly. *Circulation* 105, 2872-2877 (2002)
DOI: 10.1161/01.CIR.0000018650.58984.75

20. X Chen, W Wen, KJ Anstey, PS Sachdev: Prevalence, incidence, and risk factors of lacunar infarcts in a community sample. *Neurology* 73, 266-272 (2009)
DOI: 10.1212/WNL.0b013e3181aa52ea

21. Z Cai, W He, CY Peng, J Zhou, QL Xu, ZS Wu: The prevalence of lacunar infarct decreases with aging in the elderly: a case-controlled analysis. *Clin Interv Aging* 11, 733-738 (2016)
DOI: 10.2147/CIA.S108166

22. C Loeb, C Gandolfo, R Croce, M Conti: Dementia associated with lacunar infarction. *Stroke* 23, 1225-1229 (1992)
DOI: 10.1161/01.STR.23.9.1225

23. DA Snowdon, LH Greiner, JA Mortimer, KP Riley, PA Greiner, WR Markesbery: Brain infarction and the clinical expression of alzheimer disease: the nun study. *JAMA* 277, 813-817 (1997)
DOI: 10.1001/jama.1997.03540340047031

24. MJ Villanueva-Millán, P Pérez-Matute, JA Oteo: Gut microbiota: a key player in health and disease: a review focused on obesity. *J Physiol Biochem* 71, 509-525 (2015)
DOI: 10.1007/s13105-015-0390-3

25. EM Quigley: Gut bacteria in health and disease. *J Gastroenterol Hepatol* 9, 560-569 (2013)

26. MJ Bull, NT Plummer: Part 1: the human gut microbiome in health and disease. *J Integr Med* 13, 17-22 (2014)

27. CA Janeway Jr, P Travers, M Walport, MJ Shlomchik: Immunobiology: the immune system in health and disease. The mucosa immune system. *Garland Science* (2001)

28. G Eberl: A new vision of immunity: homeostasis of the superorganism. *Mucosal Immunol* 3, 450-460 (2010)
DOI: 10.1038/mi.2010.20

29. HM Blottière, J Doré: The gut microbiota: an integrated interactive system. In: The human microbiota and chronic disease: Dysbiosis as a cause of human pathology, Sect 1 An introduction to the human tissue microbiome. Eds: L Nibali, B Henderson, *John Wiley & Sons*, New Jersey (2016)

30. LK Wei, S Menon, LR Griffiths, SH Gan: Signaling pathway genes for blood pressure, folate and cholesterol levels among hypertensives: an epistasis analysis. *J Hum Hypertens* 29, 99-104 (2015)
DOI: 10.1038/jhh.2014.53

31. SR Williams, Q Yang, F Chen, X Liu, KL Keene, P Jacques, WM Chen, G Weinstein, FC Hsu, A Beiser, L Wang: Genomics and Randomized Trials Network; the Framingham Heart Study. Genome-wide meta-analysis of homocysteine and methionine metabolism identifies five one carbon metabolism loci and a novel association of ALDH1L1 with ischemic stroke. *PLoS Genet* 10, 1-13 (2014)
DOI: 10.1371/journal.pgen.1004214

32. LK Wei, H Sutherland, A Au, E Camilleri, LM Haupt, SH Gan, LR Griffiths. A potential epigenetic marker mediating serum folate and vitamin B12 levels contributes to the risk

of ischemic stroke. *Biomed Res Int* 2015, 167976 (2015)
DOI: 10.1155/2015/167976

33. J Lehotský, B Tothová, M Kovalská, D Dobrota, A Beňová, D Kalenská, P Kaplán: Role of homocysteine in the ischemic stroke and development of ischemic tolerance. *Front Neurosci* 10, 1-16 (2016)
DOI: 10.3389/fnins.2016.00538

34. M Rossi, A Amaretti, S Raimondi: Folate production by probiotic bacteria. *Nutrients* 3, 118-134 (2011)
DOI: 10.3390/nu3010118

35. BN Manolescu, E Oprea, IC Farcasanu, M Berteanu, C Cercasov: Homocysteine and vitamin therapy in stroke prevention and treatment: a review. *Acta Biochim Pol* 57, 467-477 (2010)

36. RS Beard, JJ Reynolds, SE Bearden: Hyperhomocysteinemia increases permeability of the blood-brain barrier by NMDA receptor-dependent regulation of adherens and tight junctions. *Blood* 118, 2007-2014 (2011)
DOI: 10.1182/blood-2011-02-338269

37. JR Kelly, PJ Kennedy, JF Cryan, TG Dinan, G Clarke, NP Hyland: Breaking down the barriers: the gut microbiome, intestinal permeability and stress-related psychiatric disorders. *Front Cell Neurosci* 9, 392 (2015)
DOI: 10.3389/fncel.2015.00392

38. V Braniste, M Al-Asmakh, C Kowal, F Anuar, A Abbaspour, M Tóth, A Korecka, N Bakocevic, LG Ng, P Kundu, B Gulyás: The gut microbiota influences blood-brain barrier permeability in mice. *Sci Transl Med* 6, 158 (2014)
DOI: 10.1126/scitranslmed.3009759

39. M Carabotti, A Scirocco, MA Maselli, C Severi: The gut-brain axis: interactions between enteric microbiota, central and enteric nervous systems. *Ann Gastroenterol* 28, 203 (2015)

40. M Jandzinski: Manipulation of the microbiome and its impact on functional recovery following ischemic stroke. *Honors Scholar Theses*, 1-41 (2015)

41. SJ Lahtinen, L Tammela, J Korpela, R Parhiala, H Ahokoski, H Mykkänen, SJ Salminen: Probiotics modulate the *Bifidobacterium* microbiota of elderly nursing home residents. *Age* 31, 59-66 (2009
DOI: 10.1007/s11357-008-9081-0

42. S Rampelli, M Candela, M Severgnini, E Biagi, S Turroni, M Roselli, P Carnevali, I Donini, PA Brigidi: Probiotics-containing biscuit modulates the intestinal microbiota in the elderly. *J Nutr Health Aging* 17, 166-172 (2013)
DOI: 10.1007/s12603-012-0372-x

43. A Everard, S Matamoros, L Geurts, NM Delzenne, PD Cani: *Saccharomyces boulardii administration* changes gut microbiota and reduces hepatic steatosis, low-grade inflammation, and fat mass in obese and type 2 diabetic db/db mice. *mBio* 5, e01011-14 (2014)
DOI: 10.1128/mBio.01011-14

44. E Akbari, Z Asemi, RD Kakhaki, F Bahmani, E Kouchaki, OR Tamtaji, GA Hamidi, M Salami: Effect of probiotic supplementation on cognitive function and metabolic status in alzheimer's disease: a randomized, double-blind and controlled trial. *Front Aging Neurosci* 8, 1-8 (2016)
DOI: 10.3389/fnagi.2016.00256

45. H Chui: Dementia due to subcortical ischemic vascular disease. *Clin Cornerstone* 3, 40-51 (2001)
DOI: 10.1016/S1098-3597(01)90047-X

46. SKhalesi, J Sun, N Buys, R Jayasinghe: Effect of probiotics on blood pressure: a systematic review and meta-analysis of randomized, controlled trials. *Hypertension* 64, 897-903 (2014)
DOI: 10.1161/HYPERTENSIONAHA.114.03469

47. JY Dong, IMY Szeto, K Makinen, Q Gao, J Wang, LQ Qin, Y Zhao: Effect of probiotic fermented milk on blood pressure: A meta-analysis of randomised controlled trials. *Br J Nutr* 110, 1188-1194 (2013)
DOI: 10.1017/S0007114513001712

48. L Seppo, T Jauhiainen, T Poussa, R Korpela: A fermented milk high in bioactive peptides has a blood pressure–lowering effect in hypertensive subjects. *Am J Clin Nutr* 77, 326-330 (2003)

49. K Aihara, O Kajimoto, H Hirata, R Takahashi, Y Nakamura: Effect of powdered fermented milk with *Lactobacillus helveticus* on subjects with high-normal blood pressure or mild

hypertension. *J Am Coll Nutr* 24, 257-265 (2005)
DOI: 10.1080/07315724.2005.10719473

50. M Gómez-Guzmán, M Toral, M Romero, R Jiménez, P Galindo, M Sánchez, MJ Zarzuelo, M Olivares, J Gálvez, J Duarte: Antihypertensive effects of probiotics Lactobacillus strains in spontaneously hypertensive rats. *Mol Nutr Food Res* 59, 2326-2336 (2015)
DOI: 10.1002/mnfr.201500290

51. GR Gibson, MB Roberfroid: Dietary modulation of the human colonie microbiota: Introducing the concept of prebiotics. *J Nutr* 125, 1401-1412 (1995)

52. S Patel, A Goyal: The current trends and future perspectives of prebiotics research: a review. *3 Biotech* 2, 115-125 (2012)
DOI: 10.1007/s13205-012-0044-x

53. P Su, A Henriksson, H Mitchell: Prebiotics enhance survival and prolong the retention period of specific probiotic inocula in an *in vivo* murine model. *J Appl Microbiol* 103, 2392-2400 (2007)
DOI: 10.1111/j.1365-2672.2007.03469.x

54. OO Adebola, O Corcoran, WA Morgan: Synbiotics: the impact of potential prebiotics inulin, lactulose and lactobionic acid on the survival and growth of lactobacilli probiotics. *J Funct Foods* 10, 75–84 (2014)
DOI: 10.1016/j.jff.2014.05.010

55. PK Gopal, J Prasad, HS Gill: Effects of the consumption of *Bifidobacterium lactis* HN019 (DR10TM) and galacto-oligosaccharides on the microflora of the gastrointestinal tract in human subjects. *Nutr Res* 23, 1313-1328 (2003)
DOI: 10.1016/S0271-5317(03)00134-9

56. L Piirainen, RA Kekkonen, K Kajander, T Ahlroos, S Tynkkynen, R Nevala, RKorpela: In school-aged children a combination of galacto-oligosaccharides and *Lactobacillus* GG increases bifidobacteria more than *Lactobacillus* GG on its own. *Ann Nutr Metab* 52, 204-208 (2008)
DOI: 10.1159/000138124

57. DD Gallaher, J Khil: The effect of synbiotics on colon carcinogenesis in rats. *J Nutr* 129, 1483S-1487S (1999)

58. S Fujimori, K Gudis, K Mitsui, T Seo, M Yonezawa, S Tanaka, A Tatsuguchi, C Sakamoto: A randomized controlled trial on the efficacy of synbiotic versus probiotic or prebiotic treatment to improve the quality of life in patients with ulcerative colitis. *Nutrition* 25, 520-525 (2009)
DOI: 10.1016/j.nut.2008.11.017

Abbreviations: SASP: Senescence-Associated Secretory Phenotype

Key Words: Microbiome, Probiotics, Aging, Dementia, Alzheimer's Disease, Lacunar Stroke, Homocysteine, Synbiotics, Review

Send correspondence to: Loo Keat Wei, Department of Biological Science, Faculty of Science, Universiti Tunku Abdul Rahman, Bandar Barat, 31900, Kampar, Perak, Malaysia, Tel: +605-4688888, Fax: +605-4671676, E-mail: wynnelkw@gmail.com