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Review

Probiotics: The Next Dietary Strategy against Brain Aging

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ABSTRACT: Owing to their long history of safe use, probiotic microorganisms, typically from the genus *Lactobacillus*, have long been recognized, especially in traditional and fermented food industries. Although conventionally used for dairy, meat, and vegetable fermentation, the use of probiotics in health foods, supplements, and nutraceuticals has gradually increased. Over the past two decades, the importance of probiotics in improving gut health and immunity as well as alleviating metabolic diseases has been recognized. The new concept of a gut-heart-brain axis has led to the development of various innovations and strategies related to the introduction of probiotics in food and diet. Probiotics influence gut microbiota profiles, inflammation, and disorders and directly impact brain neurotransmitter pathways. As brain health often declines with age, the concept of probiotics being beneficial for the aging brain has also gained much momentum and emphasis in both research and product development. In this review, the concept of the aging brain, different *in vivo* aging models, and various aging-related benefits of probiotics are discussed.

Keywords: aging, gut-brain axis, neurotransmitter, probiotic

INTRODUCTION TO AGING

Aging is defined as the linear and progressive loss of functional capability over time (calendar age). It is characterized by a complex process causing the deterioration of fitness components, such as age-related performance and productivity (Lipsky and King, 2015). Almost all organisms on earth, from bacteria to nematodes and vertebrates, are affected by aging. Not only is aging associated with declining physical performance, but it also affects mental health and cellular processes, ultimately leading to morbidity and mortality (Ferrucci et al., 2008). According to the World Health Organization (WHO), individuals with a chronological age of 65 are referred to as elderly and old (Kowal and Dowd, 2001). In 2017, approximately 962 million people worldwide were of age $\geq$60, representing 13% of the total world population (National Institutes of Health, 2016). As we age, we may experience age-associated diseases such as reduced strength and coordination and impaired vision and hearing abilities, metabolic disorders such as diabetes and cardiovascular diseases, and neurological disorders such as Alzheimer's and Parkinson's diseases (Ferrucci et al., 2008). An aging population also contributes to socioeconomic burden due to increased healthcare expenses, less labor force participation, pension costs, and poverty (National Institutes of Health, 2016). Therefore, it is essential to understand the biological processes underlying aging to develop treatments that delay aging and/or promote healthy aging. To date, aging is one of the most debated topics among scientists worldwide.

THE AGING BRAIN

Aging is closely associated with a decline in cognitive functions and other behavioral deficits such as anxiety. As per 10 population studies conducted in China, France, Italy, Korea, and the United States, the prevalence of mild cognitive impairment ranges from 3% to 42% (Ward et al., 2012). Mild cognitive impairment, a common condition in the elderly, is characterized by the deterioration of memory, attention, and cognitive function beyond that expected for a particular age and educational level (Eshkoor et al., 2015). Memory impairment is considered as a marker of Alzheimer's disease and dementia (Ritchie

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and Touchon, 2000). The prevalence of dementia in the elderly population is 1% to 2% per year (Eshkoor et al., 2015). The number of dementia cases in the population aged >60 has been postulated to increase from 9.4% (in 2000) to 23.5% (in 2050) (Eshkoor et al., 2015).

A study involving 1,248 adults aged 52 to 88 found that performance on the auditory verbal learning test (a test for learning ability) declined after the age of 58, whereas processing speed and executive function declined after the age of 50 (Li et al., 2014). The effects of aging on the brain and cognition are widespread and have multiple etiologies such as genetics, neurotransmitter pathways, hormones, and experience (Peters, 2006). Changes in brain structural connectivity, decrease in neurogenesis, lipid peroxidation, oxidative stress, mitochondrial dysfunction, decline in neurotransmitters levels, and beta-amyloid (Aβ) overproduction have also been suggested to play a major role in the etiology of brain aging and age-related neurological disorders (Luo et al., 2009; Ali et al., 2015; Li et al., 2015). Most of these etiological factors have been associated with an increase in reactive oxygen species (ROS) during aging, suggesting that ROS are the main mediators of brain aging. Impairment of DNA repair mechanisms with age has also been proposed to contribute to the development of age-related degenerative diseases (Langie et al., 2017).

CAUSES OF AGING

Through decades of research on aging, many theories related to the causes of aging have been proposed, and it became undeniable that aging is a multifactorial process. Most theories revolve around telomere shortening, oxidative stress, and energy homeostasis imbalance (Weinert and Timiras, 2003; Lipsky and King, 2015; Sergiev et al., 2015), which are all discussed in this review. Altogether, two notions have emerged—aging is either programmed in the cells on the day they are generated or it occurs through the accumulation of damage as we grow.

Telomere shortening

Telomeres are a set of highly conserved repetitive DNA sequences situated at the ends of every linear chromosome. They protect chromosomal integrity similar to the aglets on shoelaces (Weinert and Timiras, 2003; Lipsky and King, 2015). Telomeres are the main factors responsible for limiting the number of cell divisions. Every cell undergoes replicative senescence, a natural selection process through which the number of cell divisions is predetermined and limited. A part of telomeric DNA at the end of a chromosome disappears after every cell division, and as telomeres reach a critically shortened length, cells undergoes replicative senescence (Weinert and Timiras, 2003; Heidinger et al., 2012). Shortened telomeres can be repaired by the telomerase enzyme, which functions by extending telomeric DNA. However, telomerase is found only in cells with indefinite proliferating ability such as germ, stem, and cancerous cells. Majority of cells with limited cycles of cell division lack telomerase activity and, consequently, present a shortening of telomeres (Sergiev et al., 2015). Therefore, telomere length is often used as a biological clock to determine the lifespan of an organism. A study by Steenstrup et al. (2017) found that in humans, telomeres might be a major determinant of an individual's natural lifespan limit. The study evidenced that females, who live approximately 5 years longer than males according to the Human Mortality Database for 2010, usually have longer telomeres than males (150 bp difference). Another study on zebrafish also correlated the telomere length measured in early life to longevity, with fish with longer telomeres at 25 days of age tending to live longer (Heidinger et al., 2012).

Oxidative stress

Oxidative stress or free radical theory is the most discussed theory of aging requiring attention. Oxidative stress is considered the most destructive process that accelerates aging. It is prominently caused by ROS, such as highly reactive superoxide anions, hydrogen peroxide, and hydroxyl radicals (Cui et al., 2012). ROS are normally produced as by-products of metabolic reactions. Mitochondria, the powerhouse of a cell, is responsible for producing energy in the form of adenosine triphosphate and generating oxygen through the electron transport chain. During mitochondrial respiration, oxygen is consumed and reduced to form superoxide radicals and hydrogen peroxide (Cui et al., 2012; Sergiev et al., 2015). ROS are also produced in response to environmental factors including stress, radiation, chemical oxidants, inflammation, and toxins. The damaging effect of ROS and their link to aging are attributed to the ROS-induced detrimental mutations of mitochondrial DNA, DNA lesions, and rapid oxidation of lipids and proteins, which are commonly observed in aged tissues (Cui et al., 2012). Accumulation of ROS was also associated to several age-related pathologies, such as Parkinson's disease, diabetes, and cancer (Sanz, 2016). Under normal circumstances, cells produce ROS-metabolizing enzymes (antioxidants), such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase, to counteract the effects of ROS (Sergiev et al., 2015). An association between oxidative stress and aging has been further confirmed by studies using *Caenorhabditis elegans*. One study reported that a mutant strain with increased resistance to oxidative stress showed extended lifespan, and another study reported that treatment with SOD/CAT-mimicking drugs extended the organism's lifespan (Melov et al., 2000; Cui

et al., 2012). Moreover, the average maximum lifespan of transgenic *Drosophila melanogaster* with lower levels of oxidative stress was extended by up to one-third and age-related destructive changes, such as increase in the speed of walking, were reduced (Sohal and Weindruch, 1996).

Energy homeostasis imbalance

Energy homeostasis imbalance is the latest proposed cause of aging. The energy homeostasis imbalance theory suggests that energy expenditure is inversely proportional to lifespan. A natural phenomenon exists by which smaller mammals with rapid heart rates utilize more oxygen than nematodes and insects and, consequently, have shorter lifespans. Calorie restriction (CR) remains one of the most potent anti-aging interventions and best substantiates the energy homeostasis imbalance theory (Anderson et al., 2009; Lipsky and King, 2015). CR is achieved by reducing calorie intake without inducing malnutrition. In multiple models, including monkey, rat, worm, and yeast models, CR has been shown to delay aging and promote longevity (Lipsky and King, 2015). For example, in rodents, 55% to 65% CR was sufficient to show up to 65% increase in average and maximum lifespans (Anton and Leeuwenburgh, 2013). It is hypothesized that CR acts by reducing glucose and insulin levels. This in turn activates energy-sensing network regulators such as adenosine monophosphate-activated protein kinase (AMPK) and sirtuin (Anderson et al., 2009; Aliper et al., 2017). AMPK and sirtuin maintain cellular energy homeostasis by upregulating autophagy (a process through which cytoplasmic components are degraded and recycled) (Ulgherait et al., 2014). Subsequently, the removal of unwanted wastes promotes cellular health and prevents oxidative stress, thus increasing longevity (Madeo et al., 2015).

MODELS USED IN AGING RESEARCH

Investigating the aging process in humans is difficult because of their long average lifespan. Moreover, it is impossible and unethical to control factors such as diet and lifestyle, which might affect the study. Thus, various aging models that use mammals, other vertebrates, and eukaryotic microorganisms have been developed as an indispensable tools for conducting aging research. Different models have different lifespans and serve different purposes. Therefore, it is important for researchers to choose the right model according to the scope of the study, whether it is a simple genetic investigation or a more mechanistic biological study such as neuroaging research (Mitchell et al., 2015).

Nonhuman primates

In aging research, nonhuman primates and rodents are the most widely used vertebrates. Nonhuman primates are the closest animal relatives of humans, having strong resemblance to humans in terms of anatomy, genetic identity, and physiology. They experience age-related diseases such as metabolic disorders, musculoskeletal weakening, and receding fertility. The greatest advantage of using nonhuman primates over other mammals in aging research is that they have cerebral functions and thinking skills similar to those of humans and are, therefore, an effective model for neuroaging studies (Sprott, 2011; American Federation for Aging Research, 2016). Among primates such as apes, chimpanzees, and marmoset, rhesus monkeys are particularly useful as they age three times faster than humans, greatly shortening the investigation duration (American Federation for Aging Research, 2016). One of the most famous aging research on rhesus monkeys evaluated how CR delayed disease onset and mortality. In their 20-year-long longitudinal study conducted at the Wisconsin National Primate Research Center, Colman et al. (2009) found that a moderate CR ameliorates the incidence of aging-related deaths. Monkeys on normal diet developed sarcopenia (age-associated loss of muscle mass commonly seen in humans of age $\geq$60) at an early age of 15.5 (Colman et al., 2009). Another interesting finding from the same study concerned brain atrophy, which cannot be examined precisely in smaller animals. As humans age, a reduction of brain white matter at a rate of 2 mL per year is observed (Double et al., 1996). Colman et al.'s study (2009) on brain atrophy is valuable for the detection of early onsets of Alzheimer's disease; it showed that CR reduced age-associated brain atrophy in regions that control motor functions. Despite their strong resemblance to humans, the use of primates in research has some drawbacks, e.g., husbandry-related difficulties and the need of sophisticated equipment to conduct daily live monitoring. Because primates are highly intelligent animals and can potentially transmit dangerous diseases, keeping them under appropriate care is very labor and cost intensive (Mitchell et al., 2015).

Rodents

Unlike primates, rodents are easier to handle as they require smaller housing facilities. Their shorter lifespan and ease of breeding also make them the most popular experimental animals (80% of experimental animals are mice and rats). The life expectancy of rodents, especially of laboratory rats, is 3 years, which is equivalent to 90 human years, and 2-year-old rats are comparable to 60-year-old humans. The availability of these correlation data enables us to design experiments for aging research (Andreollo et al., 2012). Inbred mice are widely used for the study of aging and age-related diseases because of their genetic

uniformity. Lesser variations between animals of the same genetic strain can be produced through inbreeding. Extensive data regarding their genetics, physiology, and behavior are also available in the Mouse Phenome Database (MPD) initiated by the Jackson Laboratories in 2001. MPD contains more than 3,500 phenotypes correlating to human diseases such as cancer, obesity, and neurodegenerative disorders, which are useful for conducting mechanistic and cellular aging studies (Mitchell et al., 2015). In addition, mutant mouse strains, such as the well-known senescence-accelerated mouse (SAM) strains developed in Japan around 1970 (Takeda et al., 1991), are also readily available. In total, there are 12 mutant strains at present, namely SAM-prone (SAM-P)/1, -P/2, -P/3, -P/6, -P/7, -P/8, -P/9, and -P/10 and SAM-resistance (SAM-R)/1 and -R/2. The SAM-P and SAM-R mice present strain-specific phenotypes. For instance, senile amyloidosis in SAM-P/1, -P/2, and -P/7; degenerative joint disease in SAM-P/3; and learning and memory deficits in SAM-P/8 (Takeda et al., 1991). Other genetically modified mice developed to act as models of specific pathologies are also listed in Table 1 (Kuro-o et al., 1997; Blüher et al., 2003; Yan et al., 2007; Yuan et al., 2011; Mitchell et al., 2015).

In rodents, aging can be accelerated using chemicals such as D-galactose (D-gal). In various studies using mice and rats, chronic low-dose administration of D-gal increases oxidative stress levels and advanced glycation end-product amounts, ultimately leading to symptoms similar to those of aging. For instance, rodents aged using D-gal were found to exhibit neurological impairments, poor neuromuscular activity, and hampered immune system (Song et al., 1999). Not only aging-related genetic and biochemical changes but also the destructive effect of D-gal on cognition can be assessed in rodents with the use of behavioral tools such as the step-through method and the assessment of spontaneous motor activity, for which photocell activity cages are used to detect and record movements. Mice receiving chronic D-gal treatments displayed lower spontaneous motor activity, longer latencies, and higher error rates in the step-through method compared with naturally aged control mice (Song et al., 1999).

Fruit flies

D. melanogaster, also known as fruit fly, has been extensively used as a model in aging and longevity studies (>2,000 publications) (Hoxha, 2012). It is an attractive genetic model because of its short lifespan (the mean lifespan being 2∼3 months), easy maintenance, and genetic tractability (Sun et al., 2013; He and Jasper, 2014; Heintz and Mair, 2014). Besides, *D. melanogaster* is a relevant model for investigating age-related physiological, behavioral, and anatomical changes in humans (Iliadi et al., 2012). In general, basic approaches commonly employed in *D. melanogaster* studies include the analysis of lifespan, diet composition, food intake, lifetime reproductive output, and physiological and behavioral changes (Sun et al., 2013). Locomotor assays such as the rapid iterative negative geotaxis (RING) assay are reliable for assessing age-related physiological and behavioral changes in *D. melanogaster* (Iliadi et al., 2012; Sun et al., 2013). The RING assay measures climbing speed, locomotor activity, and escape reflex in response to mechanical stimulation. The climbing speed has been demonstrated to be age-dependent in *D. melanogaster* (Iliadi et al., 2012; Sun et al., 2013).

Furthermore, CR has been well established in *D. melanogaster* (Lee and Min, 2013) and successfully delays the process of aging and the development of age-related diseases (Taormina and Mirisola, 2014; López-Lluch and Navas, 2016). In *D. melanogaster*, CR is usually achieved by diluting nutrients, typically the protein to carbohydrate ratio (Lee and Min, 2013; Taormina and Mirisola, 2014). Moreover, more than 50% of the *D. melanogaster* genes are homologous to human genes, and more than

Table 1. Mutant mouse strains used in aging studies

Mouse strain	Description of the mutated gene	Effects of knockout	Lifespan	Reference
GHR KO	Growth-hormone-releasing hormone receptor	Abnormal development of the anterior pituitary gland	Reduced	Yuan et al. (2011)
FIR KO	Homozygous fat-specific insulin receptor	Decreased total body triglyceride levels and resistance to glucose tolerance	Increased	Blüheret al. (2003)
AC5 KO	5 Adenylyl cyclase synthesizes adenosine monophosphate from adenosine triphosphate	Enhanced femoral bone density and calcification, useful for osteoporosis study	Increased	Yan et al. (2007)
Klotho KO	Encodes a membrane protein that shares sequence similarity with β-glucosidase enzyme	Develop symptoms like aging: infertility, arteriosclerosis, skin atrophy, and osteoporosis	Reduced	Kuro-o et al. (1997)
Mutator	Mutation in the murine mtDNA polymerase	Accumulation of mtDNA mutation, weight loss, osteoporosis, and cardiomyopathy	Reduced	Mitchell et al. (2015)

GHR, growth hormone receptor; FIR, fat-specific insulin receptor; AC5, adenylyl cyclase type 5; KO, knockout; mtDNA, mitochondrial DNA.

75% of the genes involved in human diseases have fly homologs (Hoxha, 2012; Sun et al., 2013). Techniques such as mutagenesis through P-element insertion, gene expression through Gal4-unmmaned aircraft system (UAS) and GeneSwitch Gal4-UAS, and gene knockdown through RNA interference are commonly employed in aging research using *D. melanogaster* to determine single gene involvement in lifespan modulation or disease development (Sun et al., 2013). Moreover, *D. melanogaster*'s eyes have been widely used as neurodegenerative disease models to investigate Huntington's, Alzheimer's, and Parkinson's diseases (Sang and Jackson, 2005). The phenotype of the adult fly's eye is easily detected as it is tolerant to the genetic disruption of basic biological processes (Sang and Jackson, 2005).

Unicellular eukaryotes

The last aging model that we will discuss in this review is the eukaryotic budding yeast, *Saccharomyces cerevisiae*. The usage of yeast in aging research can be traced 60 years back when Mortimer and Johnston first published about yeast cells' finite replicative capacity (Mortimer and Johnston, 1959). There are two approaches for studying aging in yeast—the replicative lifespan (RLS) and chronological lifespan (CLS) (Fig. 1).

RLS refers to the number of daughter cells produced by a mother cell before reaching senescence, whereas CLS is defined as the period for which a yeast cell survives in a nondividing state (Kaeberlein et al., 2007). Being the simplest model studied, aging in yeast is affected by only two major pathways, namely the sirtuin and target of rapamycin (TOR) signaling pathways. These findings have allowed us to understand aging mechanisms and discover anti-aging therapeutic interventions in other model organisms (Longo et al., 2012). The role of sirtuin as a longevity promoter in yeast was demonstrated by RLS shortening caused by the deletion of *SIR2*, a member of the sirtuin family. Conversely, the overexpression of *SIR2* led to increased RLS. TOR signaling pathway regulates both RLS and CLS, and inhibiting TOR activity promotes longevity. Under normal growth conditions, yeasts are maintained in media supplemented with high glucose (2%) and ample amino acid concentrations. A few studies have reported that decreasing the concentration of either glucose or amino acids improves RLS and CLS (Kaeberlein et al., 2007). The drawback of using yeast as an aging model is that the homologs of yeast genes that extend RLS might not exhibit similar effects in other complex, multicellular eukaryotes. Nevertheless, it is easier to modulate and monitor variables of *in vitro* analyses in yeast than in other animal models. Yeast is also the most cost-effective model. Therefore, it still serves well to verify the hypotheses of aging, at least for the identification of some vital conserved genes.

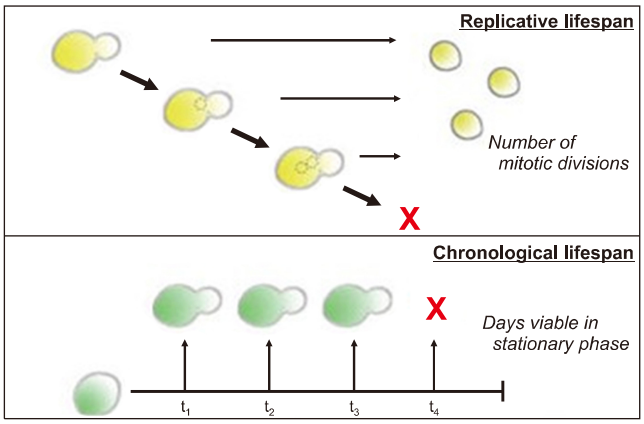

Fig. 1. Schematic illustration of the replicative and chronological lifespans of yeast aging models. Adapted from the article of Kaeberlein et al. (2007) with original copyright holder's permission.

PROBIOTICS

The intricate relationship between gut microbiota and the central nervous system has sparked great interest in using functional foods and nutraceuticals as probiotics to modulate the gut microbiome with the hope to enhance brain health (Liu et al., 2018). The definition of probiotics has undergone several revisions over the past decades. Currently, the commonly accepted definition is "live microorganisms that, when administered in adequate amounts, confer a health benefit on the host" (Food and Agriculture Organization of the United Nations, World Health Organization, 2006). The most widely studied probiotic, lactic acid bacteria (LAB), is a group of nonmotile and nonspore-forming Gram-positive bacteria. Members of the LAB family include the genera *Lactobacillus*, *Lactococcus*, *Enterococcus*, *Leuconostoc*, *Pediococcus*, and *Streptococcus*. They ferment carbohydrates and produce lactic acid as main end-product (Nair and Surendran, 2005). The probiotic effects of bifidobacteria, which are primarily Gram-positive, nonspore-forming, nonmotile, and catalase-negative anaerobic bacteria, are also studied (Tham et al., 2012). The past two decades have seen a rise in probiotic use owing to their therapeutic properties in different diseases, ranging from gut-related illnesses in which probiotics reduce the incidence of diarrhea, pain, constipation, and inflammation (Hor et al., 2018) to metabolic diseases in which probiotics improve hypertension (Yeo et al., 2009; Fung and Liong, 2010), hypercholesterolemia (Lye et al., 2017), and respiratory health (Hor et al., 2018). However, the emerging concept of gut-brain axis has further expanded the health benefits of probiotics beyond these conventional benefits (Liu et al., 2018).

THE GUT-BRAIN AXIS

The gut-brain axis is a two-way communication network comprising of the central nervous system, the sympathetic nervous system, the enteric nervous system, the hypothalamic-pituitary-adrenal (HPA) axis, and the gut microbiota (Fig. 2). It is a relatively new concept, recently adopted by the scientific community in an attempt to describe the connections and implications of the gut health on the brain health (Arck et al., 2010). The bidirectional communication between the brain and gut had already been recognized by studies in the early 19th century. One of the prime examples is the work from Beaumont who noted in 1833 an association between mood and gut function by monitoring gastric secretions through a fistula in a patient's stomach. His findings showed that an individual's emotional state can influence the functions of the gastrointestinal (GI) tract and vice versa (Beaumont, 1977). The gut microbiota is distributed throughout the human GI tract and carries out important metabolic and physiological functions for the host. Due to the bidirectionality of the gut-brain axis, various environmental stressors, such as restraint conditions and food deprivation, can cause unwanted alterations in the gut microbiota of hosts.

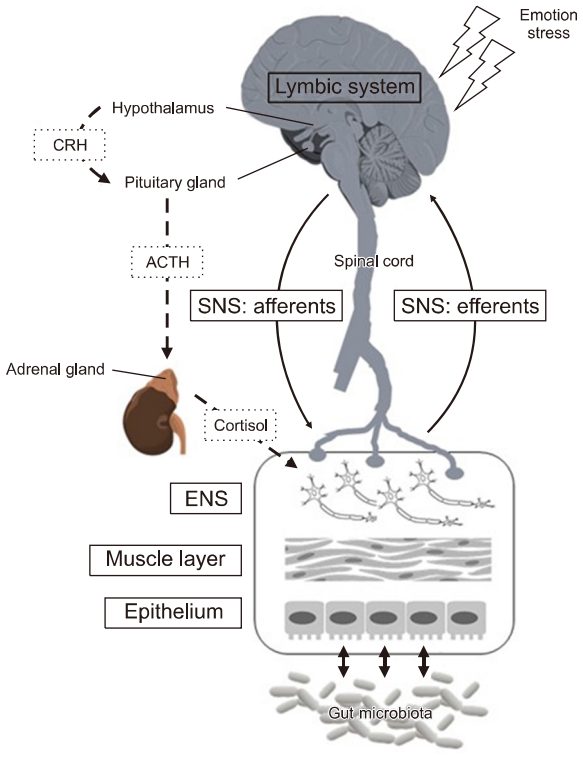

Fig. 2. Gut-brain axis structure. The hypothalamic-pituitary-adrenal (HPA) axis (in dashed lines) is activated in response to emotions or stress. Secretion of corticotropin-releasing hormone (CRH) from the hypothalamus stimulates the secretion of adrenocorticotropic hormone (ACTH) from the pituitary gland. The HPA axis ends with cortisol release from the adrenal glands. In parallel, the central nervous system communicates through both afferent and efferent sympathetic nervous systems (SNS) with different intestinal targets such as the enteric nervous system (ENS), intestinal muscle layers, and gut epithelium. There is a bidirectional communication between the gut microbiota and these intestinal targets to modulate gastrointestinal functions such as gut motility, immunity, permeability, and secretion of mucus.

GUT MICROBIOME AND THE GUT-BRAIN AXIS

The complex microbial ecosystem in the gut, also known as the gut microbiome, contains about 100 trillion microorganisms. It functions to establish the intestinal lining and contributes to its maintenance (Mangiola et al., 2016). Although the gut microbiome is composed of thousands of different microbial species, the most dominant microorganisms belong to the Firmicutes and Bacteriodetes phyla (Sartor, 2008). The microorganisms colonizing the gut are essential for maintaining mammalian health (Jarchum and Pamer, 2011) and are reported to have a symbiotic relationship with their host (Hooper and Macpherson, 2010) by interacting locally with intestinal cells and the enteric nervous system as well as directly with the central nervous system through neuroendocrine and metabolic pathways (Carabotti et al., 2015). Although still in their early stages, clinical and experimental investigations have indicated the important role of the gut microbiota in human brain development, behavior, and mood (Tillisch et al., 2013; Mayer et al., 2014).

Aging is closely associated with altered gut functions and composition. Indeed, reduced diversity of the gut microbiota and increased incidence of age-related diseases were reported in the elderly (Hor et al., 2019). Using D-gal to induce senescence in rats, Hor et al. (2019) found that the Firmicutes/Bacteroidetes ratio was significantly lowered during aging; however, this effect was prevented by the administration of probiotics such as *Lactobacillus helveticus* OFS 1515 and *Lactobacillus fermentum* DR9 (Lew et al., 2020). Moreover, *Lactobacillus paracasei* OFS 0291 and *L. helveticus* OFS 1515 reduce the amount of *Bacteroides*, which are opportunistic pathogens, whereas *L. fermentum* DR9 administration promotes the proliferation of *Lactobacillus* and consequently increases fecal acetate levels in D-gal-treated rats. Aging often causes great metabolic changes. Although probiotics ameliorate these impairments, the magnitude and specificity of the changes are often strain-dependent (Lew et al., 2020). *L. paracasei* OFS 0291 and *L. helveticus* OFS 1515 restore the levels of sugars such as arabinose and ribose to amounts similar to those present in young rats. *Lactobacillus plantarum* DR7 and *Lactobacillus reuteri* 8513d increased the fecal content of amino acids, such as tryptophan, leucine, tyrosine, cysteine, methionine, valine, and lysine, whereas the administration of *L. fermentum* DR9 led to a higher prevalence

of compounds related to carbohydrate metabolism such as erythritol, xylitol, and arabitol.

CHANGES IN NEUROCHEMICAL LEVELS

In addition to alterations of the gut sensorimotor functions, the absence of microbial colonization has been associated with neurotransmitter abnormalities in both the enteric and central nervous systems (Diaz Heijtz et al., 2011; Asano et al., 2012; Clarke et al., 2013). Brain-derived neurotropic factor (BDNF), a member of the neurotrophin family, is of particular interest because of its pivotal role in learning and memory functions. It is well established that the expression of BDNF is reduced during aging (Chapman et al., 2012). Interestingly, probiotics such as *Bifidobacterium longum* NCC3001 have beneficial effects by altering hippocampal BDNF mRNA expression (Bercik et al., 2011). In an attempt to prove the connection between probiotic and the gut-brain axis, vagotomized mice were fed *B. longum* NCC3001. Surprisingly, *B. longum* NCC3001 had no therapeutic effects in vagotomized mice, providing insights into the connection between probiotics and the gut-brain axis. Many other studies have shown the direct interaction of probiotics with receptors within the GI tract, potentially via the vagus nerve. There are increasing evidences regarding neuroactive compounds, particularly compounds involved in neurotransmitter production, produced by *Lactobacilli*. The effects of probiotics in the regulation of neurotransmitter signaling in the central nervous system can be direct and indirect (Wang et al., 2016).

Recently, Liu et al. (2020) have reported that adults receiving *L. plantarum* DR7 for 12 weeks experienced reduced stress and anxiety compared with adults in the placebo group. The administration of *L. plantarum* DR7 also induced changes in the brain neurotransmitter pathways including those of serotonin, dopamine, and norepinephrine (Liu et al., 2020). During the 12-week administration, both the alpha and beta diversities of the gut microbiota of the DR7 group were different from those of the placebo group at the class and order levels. Differences in specific bacterial groups were identified, showing consistency at different taxonomic levels including the phyla of Bacteroidetes and Firmicutes and the classes of Deltaproteobacteria and Actinobacteria. Bacteroidetes, Bacteroidia, and Bacteroidales, which had reduced abundance in the placebo group, were negatively correlated with dopamine beta hydrolase (DBH) gene expression in the dopamine pathway, whereas Bacteroidia and Bacteroidales were positively correlated with tryptophan hydroxylase-II (TPH2) gene expression in the serotonin pathway. A correlation was observed between DBH and Firmicutes, Clostridia, Clostridiales, Blautia, and Romboutsia, which had increased abundance in the placebo group. With regard to the serotonin pathway, Blautia was associated with tryptophan 2,3-dioxygenase, whereas Romboutsia was negatively correlated with TPH2. Deltaproteobacteria and Desulfovibrionales, which had reduced abundance in the placebo group, were negatively correlated with DBH, whereas Bilophila was associated with TPH2. This was a pioneer study of the gut-brain axis that not only showed the physiological changes induced by a probiotic but also the association of the gut microbiota with taxa-specific changes along the serotoninergic and dopaminergic pathways of the brain.

CHANGES IN IMMUNOMODULATORY RESPONSES

Increased expression of proinflammatory cytokines such as interferon-γ (IFN-γ) and interleukins (ILs) has been implicated in aging and age-related diseases (Michaud et al., 2013). The increase in inflammation ultimately leads to the hyperactivation of the HPA axis, altering neurotransmitter activity and metabolism. Numerous cytokine receptors are located on peripheral nerves, suggesting their involvement in initiating neuroinflammation and subsequently causing cognitive decline (Goehler, 2008; Luo et al., 2014). It has been proposed that probiotics indirectly improve the regulation of the HPA axis and neurotransmitter activity by ameliorating GI integrity and decreasing gut inflammation in hosts. The performance of probiotic-fed early-life stress (ELS) rats in the force swimming test improved and the concentration of proinflammatory cytokine IL-6 reduced (Desbonnet et al., 2010). In comparison to control rats, both the parameters were normalized in probiotic-fed ELS rats, suggesting a close relationship between probiotics and anti-inflammatory responses in improving brain health. In addition, Luo et al. (2014) demonstrated that the administration of *L. helveticus* NS8 prevents hyperammonemia-induced neuroinflammation, which subsequently alleviated cognitive impairment in rats. These studies imply that probiotics indirectly modulate brain health via immunomodulatory pathways.

Using rats in which aging was chemically induced (with D-gal), Hor et al. (2019) showed that aging causes immunological alterations in the colon. These alterations were successfully reversed by probiotic strains such as *L. paracasei* OFS 0291, *L. helveticus* OFS 1515, and *L. fermentum* DR9, typically via immunomodulatory properties such as reducing the levels of colonic proinflammatory cytokines tumor necrosis factor-α (TNF-α), IFN-γ, IL-1β, and IL-4, in D-gal treated rats but not in naturally aged controls.

The probiotic *L. plantarum* DR7 reduces stress and anx-

iety in adults. It also suppresses plasma proinflammatory cytokines such as IFN-γ and TNF-α in adults and enhances the levels of anti-inflammatory cytokines such as IL-4 and IL-10. Supplementation of DR7 were also accompanied by reduced plasma peroxidation and oxidative stress levels compared with the placebo (Chong et al., 2019). These anti-inflammatory effects too were attributed to DR7, which enhanced the activities of nonresting and mature natural killer cells without requiring as much T-cell activation as required in the placebo group in whom higher expressions of plasma cluster of differentiation (CD)44, CD117, CD4, and CD8 were found (Chong et al., 2019).

PHYSIOLOGICAL CHANGES OBSERVED DURING AGING AND THE ROLES OF PROBIOTICS

During aging, various physiological parameters are affected, leading to brain function alterations. This section discusses age-related changes involving microbiome, inflammation, and oxidative stress. The effects of these changes on brain functions and the role of probiotics in alleviating aging-related physiological effects and improving brain functions are also presented.

Gut microbiota

Aging affects various physiological parameters, which subsequently affect brain functions. Changes in gut microbiota composition have been suggested as one of the factors affecting brain functions. Microbial colonization of the GI tract begins at birth, continues to develop, and undergoes dramatic changes in the first year of life (Bäckhed et al., 2015). From its initial low diversity and complexity, the intestinal microbiota evolves to contain a diverse and complex population. Aging is often reported to be associated with dysbiosis of the gut microbiota, possibly because of decreased bowel motility in the elderly. This has a negative effect on digestion and might alter the gut microbiota. Hopkins and Macfarlane (2002) reported that *Bacteroides* diversity increases in the feces of healthy elderly people, whereas *Bifidobacterium* diversity decreases with age. Claesson et al. (2011) reported that the core microbiota of elderly subjects was distinct from that of younger adults, with a greater proportion of *Bacteroides* spp. and distinct abundance patterns of *Clostridium* groups in the elderly. It was also shown that the number of *Bacteroides* decreased, whereas *Escherichia coli* and *Enterococci* spp. increased in older individuals (Enck et al., 2009). Furthermore, it was discovered that the *Ruminococcus* group is the most predominant group in the elderly, contributing to approximately 9.6% of the total microbiota. In general, the gut microbiota of elderly subjects is characterized by a reduced bacterial diversity, shifts in the dominant species, decline in beneficial microorganisms, and an increase in facultative anaerobic bacteria (Salazar et al., 2017).

The ability of probiotics to colonize and alter gut microbiota composition has been commonly reported (Zhang et al., 2014; Kato-Kataoka et al., 2016; Zhang et al., 2018). Considering the importance of the gut microbiota in modulating brain functions, the use of probiotics to modulate the gut ecosystem and in turn improve brain functions has been increasingly investigated. Evidence of probiotic-mediated modulation of gut microbiota composition has been provided by several studies (Oh et al., 2016; Harata et al., 2017; Xue et al., 2017). A few mechanisms underlying the probiotic-mediated modulation of gut microbiota have also been proposed. For example, probiotics might exert antimicrobial activity and/or compete for mucosa-binding sites with other microorganisms, which might ultimately suppress the growth of gut pathogens (Collado et al., 2007; Spinler et al., 2008). Probiotics have also been reported to modulate intestinal immunity and barrier functions, which might lead to altered responsiveness of the intestinal epithelia and immune cells to microbes in the gut (Hemarajata and Versalovic, 2013).

Inflammation

Another main feature of the aging process is a chronic progressive increase in the proinflammatory status, which is termed as "inflammaging" (Franceschi et al., 2000). Increasing evidence has linked both peripheral inflammation and neuroinflammation to impairment of brain functions, such as cognitive decline and neurodegeneration, in the aging population. A correlation between markers of inflammation and Alzheimer's disease, Parkinson's disease, and mild cognitive impairment has been observed (Simen et al., 2011). The link between inflammatory cytokines and cognitive functions was supported by a meta-analysis study, which included 15,828 participants at baseline. Subjects with high circulating IL-6 levels were 1.42 times more likely to experience global cognitive decline over a 2 to 7-year follow-up period compared with those with low circulating IL-6 levels (odds ratio: 1.42, 95% confidence interval: 1.18∼1.70; $P<0.001$) (Bradburn et al., 2018). A case-control study involving elderly patients with major depressive disorder also showed that serum levels of IL-6 were associated with clinical characteristics of depression and cognitive decline (Ali et al., 2018). Additionally, elevated levels of the proinflammatory cytokines IL-17A and TNF-α and decreased levels of IL-10 were associated with the cognitive impairment observed in patients with multiple sclerosis (Trenova et al., 2018).

Probiotics have been proposed as potential immune

system adjuvants that might improve some of the age-related inflammatory features, thus serving as a potential therapeutic agent to ameliorate brain cognitive function in the elderly. The ability of probiotics to exert an immunomodulatory activity was shown in a randomized, double-blind, placebo-controlled trial involving 100 children with severe sepsis. Probiotics were administered to the children for 7 days and resulted in a significant decrease in proinflammatory cytokine (IL-6, IL-12p70, IL-17, and TNF-α) levels and an increase in anti-inflammatory cytokine (IL-10 and transforming growth factor-β1) levels (Angurana et al., 2018). This is further corroborated by a recent meta-analysis showing that probiotic intervention reduces the levels of IL-6 and C-reactive protein in middle-aged and older adults with chronic low-grade inflammation (Custodero et al., 2018). It was hypothesized that probiotics exert their immunomodulatory activities by improving the intestinal barrier integrity and restoring tight junction proteins in the gut (Zaylaa et al., 2018).

Oxidative stress

The role of oxidative stress in aging was first proposed in 1956 and is currently one of the most established explanations for how ageing occurs at the biochemical and molecular levels (Bokov et al., 2004). Oxidative stress is defined as the damage resulting from redox imbalance, which leads to an increase in destructive free radicals such as ROS and a reduction in antioxidants (Birch-Machin and Bowman, 2016). ROS are the by-products of mitochondrial respiration; thus, mitochondria have been suggested to be the primary target of oxidative damage (Cui et al., 2012). Studies have now shown mitochondrial dysfunction and increased oxidative damage in lipids, DNA, and proteins and correlated them with a variety of age-related diseases, such as metabolic diseases and brain function impairment. Oxidative stress has been reported to induce amyloid beta formation in the brain, which contributes to Alzheimer's disease development. Oxidative stress often leads to neuroinflammation, an important factor in the pathophysiology of neuropsychiatric diseases, including depression, Alzheimer's diseases, or schizophrenia (Popa-Wagner et al., 2013). High levels of ROS are also frequently correlated to neuronal death in various neurological disorders, such as Parkinson's or Alzheimer's disease (Guglielmotto et al., 2009). Considering that oxidative stress eventually increases the rate of cognitive impairment in the aging population, studies are now focusing on improving antioxidative defenses. For example, the administration of the polyunsaturated fatty acid, docosahexaenoic acid, or n-3 fatty acid reduces ROS accumulation, leading to enhanced neuroprotection (Kang and Gleason, 2013; Tan et al., 2016).

The antioxidative activity of probiotics has also been reported in many studies. For example, the consumption of probiotic yogurt containing *Lactobacillus acidophilus* for 9 weeks decreased the levels of oxidative stress markers (Mikelsaar and Zilmer, 2009). A randomized, double-blind, placebo-controlled clinical trial showed that the

Table 2. Clinical evidence on the psychotropic properties of *Lactobacillus* spp.

Product/strain	Subject characteristic	Study design	Main finding	Reference
Lactobacillus casei strain Shirota	Patients with chronic fatigue symptoms	Double-blind, randomized, placebo-controlled trial	Reduction in anxiety (P<0.05) evaluated by Beck anxiety inventory Increased fecal *Lactobacillus* and *Bifidobacterium* (P<0.05)	Rao et al. (2009)
Lactobacillus helveticus R0052	Healthy subjects, 30~60 years old	Double-blind, randomized, placebo-controlled trial	Reduction in self-reported stress (P<0.05) evaluated by perceived stress scale Reduction in urinary free cortisol (P<0.05)	Messaoudi et al. (2011)
Lactobacillus bulgaricus and *Lactococcus lactis* subsp. *lactis*	Healthy subjects, 18~55 years old	Single-center, randomized, controlled, parallel-arm trial	Reduced task-related response (P<0.004) evaluated by emotional attention task	Tillisch et al. (2013)
L. helveticus-fermented milk	Healthy subjects 60~75 years old	Double-blind, randomized, placebo-controlled trial	Improved memory performances (P<0.05) evaluated by digit span test and story recall test Improved attention performance (P<0.05) evaluated by rapid visual information-processing	Chung et al. (2014)
Lactobacillus acidophilus and *L. casei*	Major depressive disorder patients, 20~55 years old	Double-blind, randomized, placebo-controlled trial	Reduction in depression (P<0.001) evaluated by Beck depression inventory	Akkasheh et al. (2016)
L. acidophilus, *Lactobacillus fermentum*, and *L. casei*	Alzheimer's disease patients, 60~95 years old	Double-blind, randomized, placebo-controlled trial	Improved cognition evaluated by Mini-Mental State Examination (P<0.001) Increased malondialdehyde plasma levels (P<0.001) Increased high-sensitivity C-reactive protein serum levels (P<0.001) Increased beta cell function (P<0.001)	Akbari et al. (2016)

levels of oxidative stress biomarkers, including plasma total antioxidant capacity and total glutathione (GSH), in patients with type 2 diabetes are ameliorated by the administration of multispecies probiotics (Asemi et al., 2013). The administration of *L. plantarum* P-8 in rats fed high-fat diets has beneficial effects against high-fat diet-induced oxidative stress, as reflected by the decreased accumulation of liver lipids and protection of liver functions (Bao et al., 2012). A few mechanisms of probiotic antioxidative activity have also been proposed. As discussed and reviewed by Wang et al. (2017), probiotics exert an antioxidative activity by chelating metal ions and thus preventing metal ions from catalyzing oxidation. Probiotics also increase the activity of antioxidant enzymes such as SOD, which catalyzes the breakdown of superoxide ions into hydrogen peroxide and water and is therefore a central regulator of ROS levels (Landis and Tower, 2005). Probiotics also produce antioxidant metabolites such as GSH and folate (Kullisaar et al., 2002) and stimulate several host pathways related to antioxidative activities (Wang et al., 2017). Finally, probiotics might exert their antioxidative role by altering gut microbiota composition, which might prevent the excessive proliferation of harmful bacteria that contribute to oxidative stress (Wang et al., 2017).

PSYCHOBIOTICS

In recent years, there has been a growing research interest in targeting the gut microbiome to beneficially impact brain health. A promising strategy is the use of psychobiotics. Psychobiotics are beneficial bacteria, particularly probiotics, or support for these bacteria (prebiotics) that, when consumed in adequate quantities, influence bacteria-brain relationships (Dinan et al., 2013). The therapeutic effects of psychobiotics are often categorized into: (1) systemic effects on stress response, most notably on the HPA axis; (2) physiological changes in neurotransmitters and neural proteins; and (3) psychological changes including changes in emotional and cognitive functions (Sarkar et al., 2016). The translation of the psychotropic properties of probiotics observed in rodents to humans has been surprisingly promising. In the past decade, numerous clinical studies on the use of probiotics as putative psychobiotics by elderly subjects have been carried out (Table 2) (Rao et al., 2009; Messaoudi et al., 2011; Tillisch et al., 2013; Chung et al., 2014; Akbari et al., 2016; Akkasheh et al., 2016). For instance, cognitive fatigue was improved in healthy older adults treated with *L. helveticus* IDCC3801-fermented milk (Chung et al., 2014). Another recent study showed the ability of probiotics in improving the cognitive function of patients with Alzheimer's disease (Akbari et al., 2016). Patients provided probiotic milk for a short period of 12 weeks showed a remarkable 35% improvement in cognitive functions as assessed by the Mini-Mental State Examination score. These studies provide evidences of the promising therapeutic effects of probiotics on cognition, especially during aging.

CONCLUSION

The increasing consumer demand for nutraceuticals and health foods has prompted much innovation and developments in the field of probiotics, including the development of new perspectives on the link between brain health and the gut-brain axis. More importantly, a decline in brain health is also often associated with aging, and a healthy brain ensures that the elderly are able to live independently. Here, we present crucial new findings regarding the roles of probiotics in healthy aging and the preservation of brain and gut wellbeing. Additionally, we demonstrated the potential of probiotics as a natural anti-aging dietary strategy.

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AUTHOR DISCLOSURE STATEMENT

The authors declare no conflicts of interest.

AUTHOR CONTRIBUTIONS

Writing the article: JSO, LCL, YYH. Critical review of article: MTL. Overall responsibility: MTL.

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