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Review

Gerobiotics: probiotics targeting fundamental aging processes

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Aging is recognized as a common risk factor for many chronic diseases and functional decline. The newly emerging field of geroscience is an interdisciplinary field that aims to understand the molecular and cellular mechanisms of aging. Several fundamental biological processes have been proposed as hallmarks of aging. The proposition of the geroscience hypothesis is that targeting holistically these highly integrated hallmarks could be an effective approach to preventing the pathogenesis of age-related diseases jointly, thereby improving the health span of most individuals. There is a growing awareness concerning the benefits of the prophylactic use of probiotics in maintaining health and improving quality of life in the elderly population. In view of the rapid progress in geroscience research, a new emphasis on geroscience-based probiotics is in high demand, and such probiotics require extensive preclinical and clinical research to support their functional efficacy. Here we propose a new term, "gerobiotics", to define those probiotic strains and their derived postbiotics and para-probiotics that are able to beneficially attenuate the fundamental mechanisms of aging, reduce physiological aging processes, and thereby expand the health span of the host. We provide a thorough discussion of why the coining of a new term is warranted instead of just referring to these probiotics as anti-aging probiotics or with other similar terms. In this review, we highlight the needs and importance of the new field of gerobiotics, past and currently on-going research and development in the field, biomarkers for potential targets, and recommended steps for the development of gerobiotic products. Use of gerobiotics could be a promising intervention strategy to improve health span and longevity of humans in the future.

Key words: geroscience, anti-aging, biomarkers, probiotics, gerobiotics

INTRODUCTION

The world population is aging rapidly. Life expectancy is 72 years old globally, mainly due to medical advances that reduce mortality, while the number of people aged 65 and over accounts for 8.9% of the total population. The global health span, defined as the time in life that an individual is in a reasonably good health condition, is around 63.3 years [1]. Thus, for the last 8.7 years of life, elderly people tend to experience adverse health conditions, as medical treatments fall short of improving overall health. According to the National Council on Aging, approximately 80% of senior adults have at least one chronic disease, and 77% have at least two [2]. Heart disease, stroke, cancer, and diabetes are among the most common chronic health conditions of seniors, which account for two-thirds of deaths each year. The mental health of seniors is also an important health issue. Approximately 15% of adults aged 60 and over suffer from mental disorders, such as dementia and depression. Indeed, population aging, described as the "Silver Tsunami", is bringing crises to every aspect of our society, especially to the public healthcare system. It is only logical that aging is recognized as a common risk factor for many chronic diseases and functional decline. One of the frequently asked questions about aging is, what is the role of aging in driving age-related chronic diseases and loss of function, and vice versa? On the other hand, the newly emerging field of "geroscience" asks the same question from a different perspective: What are the basic biological mechanisms responsible for the aging process and the accompanying age-related diseases or conditions [3–6]? Geroscience is an interdisciplinary field that aims to understand the relationship between aging and age-related diseases and to explore how to slow down the progression of age-related diseases and functional losses [3–6]. A geroscience approach to anti-aging interventions targeting fundamental biological mechanisms of aging could result in postponement of the onset of multiple age-related chronic diseases all at once and effectively expand health span with greater efficiency. Consequently, a reduction in

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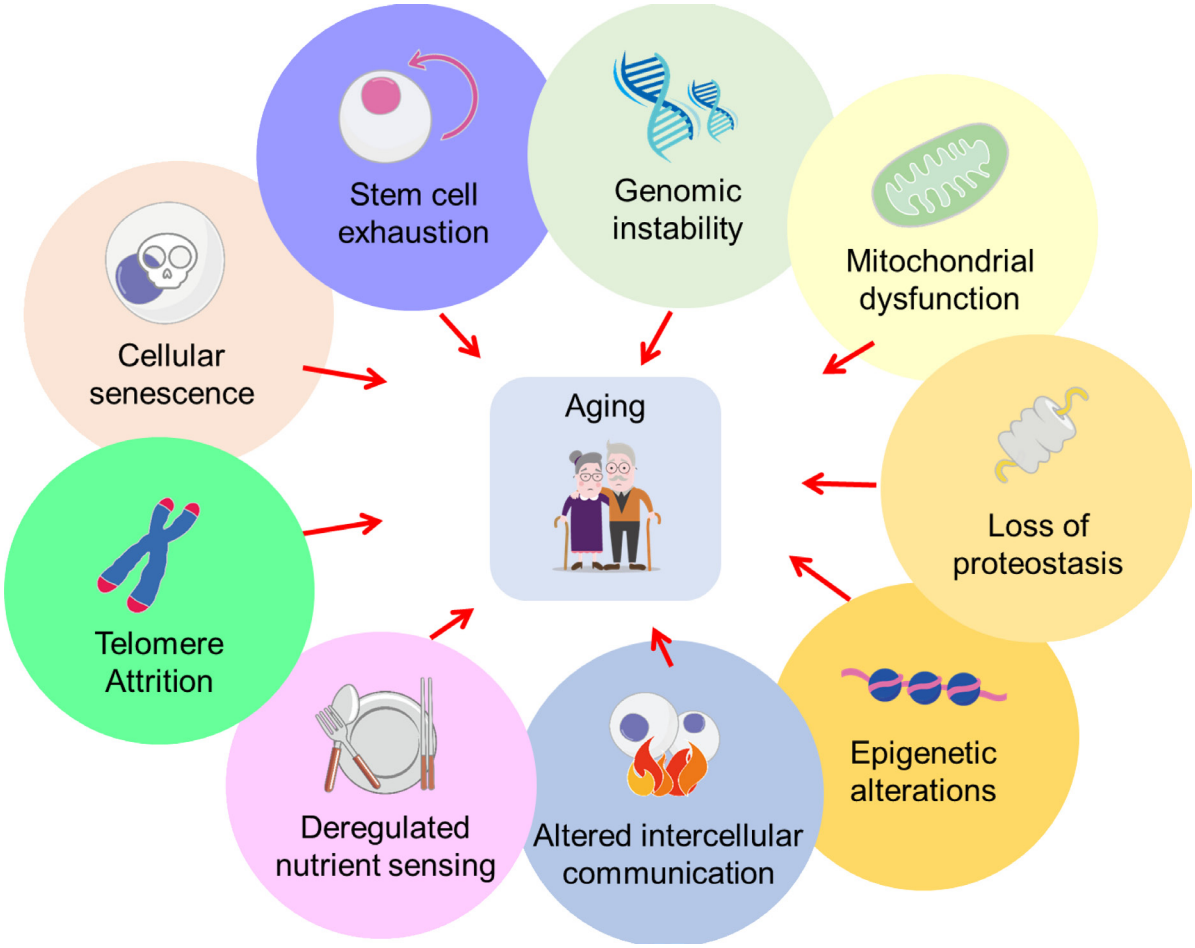

Fig. 1. Nine hallmarks of aging: genomic instability, epigenetic alterations, loss of proteostasis, telomere attrition, deregulated nutrient sensing, cellular senescence, stem cell exhaustion, mitochondrial dysfunction, and altered intercellular communication.

healthcare costs is to be expected. It is indeed a paradigm shift in our endeavor to conduct aging research. Aging not only could be postponed and slowed down but could also be reversed, so long as it is managed with the right approaches. More important for aging management is the awareness of starting it as early as possible. In the past, it was only in their 50s and 60s that people became aware of age-related health issues when signs of loss of vigor or the presence of high blood pressure, high blood sugar, or high cholesterol levels surfaced. Furthermore, only then did they start to pay attention to their diets and lifestyles and take prophylactic medication. In contrast, the concept of geroscience aims to emphasize the importance of early intervention when people are in their 40s, or even their 30s, before the appearance of symptoms of aging. This is a recent and novel concept in preventive medicine.

The rapid advancement in our understanding of the aging process has identified several fundamental biological processes as hallmarks of aging, including telomere attrition, genomic instability, epigenetic alterations, loss of proteostasis, deregulated nutrient sensing, cellular senescence, stem cell exhaustion, mitochondrial dysfunction, and altered intercellular communication (chronic inflammation; Fig. 1) [3, 7–9]. The proposition of the geroscience hypothesis is that targeting holistically these highly integrated hallmarks of aging could be an effective approach to preventing/delaying the pathogenesis of age-related diseases or functional decline simultaneously, thereby improving the health span of most individuals [10–12].

CURRENT STATUS OF EFFORTS ON GEROSCIENCE GLOBALLY

There have been more and more organizations actively investing in advancing geroscience research. The National Institute on Aging (NIA) has a mission to improve the health and well-being of the elderly population in the U.S. through biomedical, social, and behavioral research. Since 1974, the NIA has been focusing on understanding the dynamics of aging, improving the health, well-being, and independence of adults as they age, and supporting the research community. The NIA"s Interventions Testing Program (ITP) looks to test chemicals for the purpose of extending longevity [13]. Results obtained from ITP studies showed that rapamycin (both genders) [14], acarbose (both genders) [15], aspirin (males) [16], Protandim® (males) [17], nordihydroguaiaretic acid (males) [16], and glycine [18] effectively increased lifespan in a mouse model. Chemicals targeting the aging-related hallmarks are reported to not only extend lifespan but also to slow down the aging process.

In 2012, the GeroScience Interest Group (GSIG) was formed

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to explore the intersection between aging biology and the biology of diseases, with 20 NIH Institutes and Centers participating. The GSIG is focused on basic biology, together with a vision toward translation. By developing a collaborative framework, the GSIG rapidly and effectively integrated all aspects of geroscience knowledge, from basic research to clinical studies [19]. In 2013, the GSIG debuted a geroscience summit, which attracted an audience from academia, industry, and also the general public to the emerging field of geroscience, on the important public health role of geroscience in combating aging-related problems.

Rapamycin, a potent immunosuppressive agent, was known to play the key roles in cellular growth and lifespan via its targets, the nutrient-sensing protein complex and mTOR [20–22]. Resveratrol is a well-known antioxidant nutraceutical that has been reported to be an activator of sirtuins, essential factors that delay cellular senescence [23]. Cellular senescence resulting in a senescence-associated secretory phenotype (SASP), such as stem cell depletion and chronic inflammation, has been reported to be blocked by senolytics [24, 25]. Precursors of NAD^+ possibly extend longevity via activation of the NAD^+/SIRT1 axis [26]. Metformin is a widely used medication approved by the Food and Drug Administration (FDA) for treating type-2 diabetes. Studies have shown that metformin can target a number of aging-related mechanisms, such as mitochondria dysfunction, inflammation, deregulated nutrient sensing, and cellular senescence [27–29].

Recently, a large-scale human clinical geroscience trial, Targeting Aging with metformin (TAME), managed by the American Federation for Aging Research (AFAR) was designed to test the anti-aging effect of metformin on the elderly. This randomized, placebo-control trial will recruit 3,000 seniors who have slow gait speeds or age-related diseases and are between the ages of 65 and 80. The time to incidence of any age-related disease is set as the primary outcome, the time to incidence of disability is set as the secondary outcome, and changes in blood-based biomarkers of aging are set as the tertiary outcomes [9, 12, 30]. AFAR believes that the TAME trial will provide a proof of concept to test the geroscience hypothesis in humans. One of the important goals of the TAME trial is to seek FDA's recognition of aging as an indication, which will subsequently speed up the development of new treatments for a whole range of age-related diseases.

Since aging is currently not recognized as a disease, it cannot be used as an indication in clinical trials for any new drugs or new therapies [31, 32]. However, in the 2018 version of the International Classification of Diseases (ICD-11), the World Health Organization (WHO) implemented an extension code for "ageing-related" (XT9T) diseases. It is defined as those "caused by pathological processes which persistently lead to the loss of an organism's adaptation and progress in older ages". The new code "ageing-related" can be added to any other conditions listed in ICD-11, such as gastrointestinal tract dystrophy (ageing-related gastrointestinal tract dystrophy), low-grade inflammation (ageing-related low-grade inflammation), and mental disorder (ageing-related mental disorder). The new coding of aging-related diseases reveals the growing recognition of aging as a pathological process. Furthermore, it is an important move that creates more opportunities for the development and registration of innovative anti-aging biomedical technologies, identifying innovative therapies, and establishing health-promoting strategies for treating aging.

There is an increasing awareness of the benefits of prophylactic use of probiotics in maintaining health and improving quality of life in the elderly population [33–36]. As summarized in Table 1, some specific probiotic strains have been reported to have beneficial health effects in several preclinical studies using various aging animal models and in clinical studies for seniors. Although most of the proposed health-promoting mechanisms of those probiotics are immunomodulation, anti-inflammation, microbiota modulation, etc., there are a few unique strains showing the potential to affect some hallmarks of aging, such as mitochondria dysfunction (*Lactobacillus paracasei* PS23 [37], *Bifidobacterium breve* B-3 [38]) and telomere attrition (*Lactobacillus fermentum* DR9 [39]).

PROBIOTICS FOR ANTI-AGING REPORTED FROM RESEARCH MODELS

A growing body of evidence has revealed that the aging process is also tightly linked to the homeostasis of the intestinal microbiome [40–43]. Over a century ago, Élie Metchnikoff proposed that the onset of the aging process could be delayed by manipulating the intestinal environment with friendly bacteria [44]. He has been regarded as one of the pioneers of a rapidly growing field focusing on the health benefits of probiotics. Probiotics are well recognized as safe and have significant health benefits to the elderly, such as boosting immunity and maintaining gut microbiota in a healthy state. Today, probiotics are not only an important subject in the field of geroscience research but also a source of a multi-billion-dollar industry globally. Advancements in geroscience make us wonder whether certain probiotics might target basic biological mechanisms of aging. If such mechanisms can be validated, those probiotics could possibly be used to delay the onset of multiple age-related chronic diseases and thus effectively expand the health span [33].

A good number of probiotics with anti-aging potential have been identified using mainly invertebrate organism models for studying lifespan extension and rodent models for studying detailed molecular mechanisms. Some clinical trials for certain probiotic strains have even been completed with promising results with respect to their anti-aging effects.

Invertebrate model organisms

The nematode worm *Caenorhabditis elegans* and the fruit fly *Drosophila melanogaster* have been used for decades for studying anti-aging and prolongevity [45–47]. The nematode *C. elegans* is a powerful *in vivo* model in the study of host-probiotic interactions [48, 49]. In 2007, Ikeda *et al.* used *C. elegans* to test the effects of lactic acid bacteria on the lifespan of *C. elegans* and reported that *Lactobacillus rhamnosus* NBRC14710 [50] was able to improve lifespans in wild-type worms. In *C. elegans*, DAF-16 is a key transcription factor that integrates multiple signaling pathways, such as the insulin/IGF-1 signaling pathway, TOR signaling, AMPK pathway, and JNK pathway, which are involved in aging and longevity. Several probiotic strains directly or indirectly mediated by DAF-16 were reported to increase the lifespan of the worms, such as *Bifidobacterium longum* BB68 [51], *Lactobacillus gasseri* SBT2055 [52], *L. fermentum* MBC2 [53], and *Bifidobacterium infantis* ATCC15697 [54]. *Bacillus subtilis* PXN21 inhibits α-synuclein aggregation, which is also partly mediated by DAF-16 [55]. SKN-1 is yet another protein that plays important roles in oxidative stress defense and aging

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Table 1. Selected probiotic strains as potential gerobiotics

Strain	Model	Aging hallmark	Physiology	Mechanisms
B. longum BB68	*C. elegans*		Lifespan ↑	DAF-16
L. gasseri SBT2055	*C. elegans*		Lifespan ↑	p38 MAPK, SKN-1
L. fermentum MBC2	*C. elegans*		Lifespan ↑	DAF-16, pep-1
B. infantis ATCC15697	*C. elegans*		Lifespan ↑	p38 MAPK, Insulin/IGF-1, SKN-1
B. subtilis PXN21	*C. elegans*		Lifespan ↑	α-Synuclein, DAF-16
L. brevis OW38	Aged mice		Learning, memory ↑	p16, p53, SAMHD1, BDNF, DCX
L. paracasei PS23	SAMP8 mice		Senescence appearance ↓ Cognition ↑, Sarcopenia ↓	Dopamine, Serotonin, BDNF, mtDNA, PGC1-a, NRF1, TFAM, SOD, GPx, IL-6, TNF-α, MCP-1
L. paracasei K71	SAMP8 mice		Cognition ↑	MAOA, BDNF, CREB
L. plantarum AR501	D-gal mice		Liver damages ↓	Nrf2, glutathione reductase, glutathione S-transferase
L. helveticus KLDS1.8701	D-gal mice		Gut microbiota	Nrf2 , ROS, Butyrate, endotoxin
L plantarum C29	D-gal, 5XFAD mice Fischer 344 rats		Memory ↑ Gut microbiota	DCX, BDNF, p-CREB, COX-2, p-p65, iNOS, SIRT-1, p16, p53
L. plantarum NDC 75017	D-gal rats		Learning, memory↑	Mitochondrial ultrastructure, ATP level
L. fermentum DR9	D-gal rats		Exercise capacity ↑	Telomere length, p53, TNF-α, IFN-γ, IL-1β
B. lactis HN019	Mice human		Innate immunity ↑	Phagocytosis activity
B. breve B-3	Mice Rats human		Grip strength ↑ Mental condition↑ Body mass index↓ Bowel movement↑	Fasting glucose, insulin, Akt, AMPK
L. casei Shirota	human		Innate immunity ↑ Constipation ↓ Hypertension ↓	NK cells activity, IL-12

Markers of aging: 1. Altered intercellular communication. 2. Cellular senescence. 3. Deregulated nutrient sensing. 4. Genomic instability. 5. Mitochondrial dysfunction. 6. Telomere attrition. 7. Gut microbiota. 8. Neuropeptides. 9. Oxidative stress. D-gal: D-galactose, SAMP8: Senescence Accelerated Mouse-Prone 8, MAPK: mitogen-activated protein kinase, NRF1: Nuclear Respiratory Factor 1, TFAM: transcription factor A, mitochondrial, SOD: superoxide dismutase, MCP-1: Monocyte chemoattractant protein-1, DCX: Doublecortin, NK: natural killer, AMPK: AMP-activated protein kinase, CREB: cAMP-response element binding protein, SAMHD1: SAM And HD Domain Containing Deoxynucleoside Triphosphate Triphosphohydrolase 1.

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processes of *C. elegans*. [56]. The effects of extending the lifespan of *C. elegans* with *L. gasseri* SBT2055 and *B. infantis* ATCC15697 were found to be associated with the expression of SKN-1. Connections with antioxidation effects have been reported for almost all lactobacilli reported to have prolongevity potential. These lactobacilli include *L. gasseri* SBT2055, as well as several others not included in Table 1, such as *L. rhamnosus* CNCM I-3690 [57], *Lactobacillus salivarius* FDB89 [58], and *L. fermentum* LA12 [59].

D. melanogaster was rarely used for investigating anti-aging effects of probiotics in the past, but this has changed, as axenic *Drosophila* can be generated rather easily and investigating the monoassociation of individual probiotic strains has become more accessible in conducting aging-related research [60]. Recently, Gómez *et al.* suggested that the axenic Drosophila system is suitable in preliminary testing for investigating the effects of probiotics on developmental or behavioral aspects [61].

Rodent models

Rodent models of aging are usually used for investigating the anti-aging effects of probiotics. Normally aged mouse models are the closest rodent models of aging to the progression of biological aging in humans; however, the generation of such models are a time-consuming process. Oral administration of *Lactobacillus brevis* OW38 to aged mice (18 months old) for 8 weeks suppressed the expression of several senescence markers, p16, p53, and sterile α-motif domain- and HD domain-containing protein 1 (SAMHD1), in the colon and hippocampus [62]. The senescence-accelerated mouse (SAM) model was established through phenotypic selection from a common genetic pool of the AKR/J strain of mice [63]. The SAM starts senescence at approximately 6 months of age with the irreversible advancement of senescence. Among the 9 different substrains of the SAM model (SAMP1 to SAMP9), SAMP8 was most widely used for anti-aging studies of probiotics [64, 65]. *L. paracasei* PS23 preserved mitochondrial function, one of the central hallmarks of aging, and extenuated sarcopenia progression and cognitive decline with aging in SAMP8 mice [37]. Long-term administration of *L. paracasei* K71 resulted in increased protein expression of brain-derived neurotrophic factor (BDNF) and cAMP-response element-binding protein (CREB) phosphorylation in the hippocampus [66].

The D-galactose-induced accelerated aging rodent model is also widely used for evaluating anti-aging effects of probiotics. Over-administration of D-galactose can induce the generation of reactive oxygen species (ROS), oxidative stress, and inflammation and accelerate such aging processes as memory and learning deficits [67, 68]. The nuclear factor erythroid 2-related factor 2 (Nrf2)-linked pathways are involved in protective mechanisms against oxidation and have been associated with both aging and the pathogenesis of many chronic diseases. By using the D-galactose-induced aging model, it was revealed that *Lactobacillus plantarum* AR501 greatly elevated the gene expression of Nrf2 and upregulated the expressions of several antioxidant genes, such as glutathione reductase and glutathione S-transferase, in the liver of aging mice [69]. *Lactobacillus helveticus* KLDS1.8701 supplementation also reduced hepatic oxidative stress by modulating the Nrf-2 pathway [70]. Treatment with *L. plantarum* C29 ameliorated D-gal-induced suppression of BDNF and activation of CREB and decreased the expression of the senescence marker p16 and inflammation markers p-p65, cyclooxygenase (COX-2), and inducible NO synthase (iNOS) [71]. *L. plantarum* NDC 75017 treatment improved mitochondrial ultrastructure functions, including the mitochondrial respiratory chain, mitochondrial membrane potential, and mitochondrial permeability transition, in D-galactose-treated mice [72]. In a recent report, *L. fermentum* DR9, *L. plantarum* DR7, and *Lactobacillus reuteri* 8513d reduced telomere shortening induced by D-galactose treatment [39].

Human studies

Interventional studies of probiotics in the elderly population remain scarce [35], and this is primarily attributed to the challenges of a placebo control when elderly are considered a vulnerable population with comorbid conditions. In addition, it is less feasible to observe longitudinal effects of probiotics over prolonged periods of time. Thus, most aging studies in human clinical trials have involved observations of beneficial effects related to inflammation, infection, immune modulation, metabolic profiles, cognitive function, gut microbiota, and quality of life. For example, consumption of a probiotic drink containing *Lactobacillus casei* Shirota for 4 weeks by the elderly was reported to improve the tumoricidal activity of natural killer (NK) cells, increase the serum level of interleukin (IL)-10 [73], and reduce the number of days on which fever was detected [74]. Daily consumption of *Bifidobacterium lactis* HN019 for 3 or 6 weeks enhanced the phagocytic capacity of NK cells and polymorphonuclear (PMN) cells [75]. Consumption of *L. plantarum* C29 fermented soybean for 12 weeks by the elderly with mild cognitive impairment showed improvements in cognitive functions and increased serum BDNF levels [76].

Several probiotics have shown convincing evidence of anti-aging effects in a series of animal and human studies. Here we take *L. plantarum* C29, *L. paracasei* PS23, and *B. breve* B-3 as examples to illustrate the models used to study their effects on anti-aging.

***L. plantarum* C29** has been reported to improve memory impairment in various rodent models, such as in the scopolamine-induced memory deficit mouse model [77], the D-galactose-induced aging mouse model [71], the Fischer 344 rat model [78], and the TNBS-induced colitis mouse model [79]. Increased expression of BDNF, DCX, and CREB in the hippocampus; reduced inflammation markers, p-p65, p-FOXO3a, COX-2, and iNOS; and decreased senescence markers, p16 and p53, were observed with *L. plantarum* C29. In aged Fischer 344 rats, *L. plantarum* C29 treatment inhibited aging-associated activation of Akt, mTOR, and FOX3a in the colon and the hippocampus more potently than treatment with rapamycin, an mTOR inhibitor [78]. Recently, a multi-center, randomized, double-blind, placebo-controlled clinical trial was carried out to study 100 elderly subjects with an average age of 69. After consumption of *L. plantarum* C29-fermented soybean for 12 weeks, improvements in the combined cognitive functions related to memory and attention, especially in the attention domain, were observed along with increased serum BDNF levels [76].

***L. paracasei* PS23** has been shown to reduce the scores of senescence and attenuate age-related decreases of muscle mass and strength, cognitive decline, and memory impairment in the SAMP8 aging mouse model [37]. Also, it was observed that PS23 extenuated sarcopenia progression during aging, which might have been caused by the preservation of mitochondrial function

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via a reduction in age-related inflammation and ROS and by maintenance of protein uptake. Administration of PS23 increased the number of mitochondria and the expression of PGC1α, SIRT1, NRF1, and TFAM involved in mitochondrial biogenesis. Moreover, increased levels of superoxide dismutase, glutathione peroxidase activities, and tumor necrosis factor (TNF)-α and lower levels of IL-10 in serum suggested that *L. paracasei* PS23 enhanced the antioxidative capacity and modulated inflammation [80].

Based on results from the maternal separation– and chronic corticosterone treatment-induced depression mouse models, *L. paracasei* PS23 is also a potential psychobiotic [81, 82]. Administration of either live or heat-killed *L. paracasei* PS23 improved anxiety and depression-like behaviors and increased dopamine and 5-HT levels in the hippocampus and prefrontal cortex, which was accompanied by higher serum anti-inflammatory IL-10 levels and lower corticosterone levels. The anti-aging effects of heat-killed *L. paracasei* PS23 were not evaluated in SAMP8 mice; however, in the D-galactose-induced aging model, heat-killed *L. paracasei* PS23 showed improvements in muscle strength and other aging biomarkers (article in preparation) as effective as those with live cells of *L. paracasei* PS23.

***B. breve* B-3** showed anti-obesity effects in a high-fat diet-induced obesity mouse model [83] and in a clinical study on subjects with mild obesity [84]. In another clinical study on healthy elderly subjects who were supplemented with a combination probiotic containing *B. breve* B-3 and assigned to moderate resistance training, the results showed that the combination probiotic containing *B. breve* B-3 improved mental condition, body weight, and bowel movement frequency [85]. Administration of both live and heat-killed *B. breve* B-3 to 8-week-old SD rats increased muscle mass and muscle strength, promoted oxidative muscle fiber composition, and modified metabolic functions, and the effects possibly occurred via the Akt and AMPK pathways [38]. In these studies, heat-killed *B. breve* B-3 showed equivalent or even stronger effects compared with live cells. A similar phenomenon was also observed with *L. paracasei* PS23.

GEROBIOTICS: WHY A NEW TERM?

According to a consensus statement made by the International Scientific Association for Probiotics and Prebiotics (ISAPP), some mechanisms of action, such as inhibition of pathogens, bile salt metabolism, and neutralization of carcinogens, are prevalent among various probiotic strains [86]. On the other hand, some mechanisms, including neurological effects, immunological effects, and endocrinological effects, are more likely to be strain specific. Those specific probiotic strains that promote health effects by driving mucosal immune mechanisms were named "immunobiotics" [87], and those that influence brain cells and neuron cells were named "psychobiotics" [88]. These two terms are now well recognized within the probiotics research community.

Here, we propose a new term, "gerobiotics", to define those specific probiotic strains that are able to reduce physiological aging processes and those probiotic strains and their derived postbiotics and para-probiotics that are able to beneficially attenuate the fundamental mechanisms of aging, reduce physiological aging processes, and thereby expand the health span of the host.

The ISAPP, however, advises the use of existing terms for probiotics with appropriate modifiers, e.g., immune-active probiotic instead of immunobiotic and probiotic drugs instead of live biotherapeutic products [89]. Why do we now wish to create the new term gerobiotics for those anti-aging probiotic strains that show solid scientific data supporting their anti-aging mechanisms? The reasons are as follows.

In response to the rapid development of geroscience

Geroscience is a novel concept that emphasizes the importance of targeting basic mechanisms of aging for the prevention of age-related chronic diseases. This new trend caused the NIH to form the Trans-NIH Geroscience Interest Group in order to effectively integrate collaborative research [19]. It has also led WHO to implement an extension code for "ageing-related" diseases in the latest version of the ICD, which might open the door to recognition of aging as a disease and could be used as an indication in official clinical trials for new drugs or new therapies. This has not only led organizations to recognize the potential of geroscience but also drawn the attention of scientists to re-examination of the anti-aging effects of some classical drugs, such as metformin and rapamycin. Consequently, this has made the development of geroprotectors a highly coveted territory for many pharmaceutical companies [90]. Since probiotics have long been recognized as an important preventative measure for many age-related conditions and for maintenance of the general health of the elderly, we believe that a new term is needed to accelerate the development of unique probiotic strains capable of promoting health through direct effects on the fundamental mechanisms of aging.

Changing market strategies for the development of new anti-aging probiotics

It is essential for customers to experience beneficial effects personally from the probiotic products that they consume before they consider re-ordering. Unlike those probiotics for improving gastrointestinal functions and those for alleviating allergies, the beneficial effects of which users can experience within a few weeks or months, gerobiotics that aim to slow the aging process could take years or even more than ten years before noticeable anti-aging effects can be observed. New marketing strategies that are different from traditional approaches are needed for gerobiotics. In the research and development process, implementation of a panel of blood-based biomarkers that reflects the status of aging before and after interventions may serve as an effective approach to gaining the trust of those who take gerobiotics. However, the new term is not merely for implementing new market strategies effectively. It communicates well to users that certain biological markers affected by gerobiotics are actually associated with aging and aging processes.

BIOMARKERS ARE CRUCIAL FOR IDENTIFYING AND EVALUATING GEROBIOTIC CANDIDATES

Studies on the prolongevity effects of gerobiotic candidates are extremely lengthy and costly. Therefore, biomarkers could serve as much-needed indicators for evaluating interventions and providing evidence for halting of aging processes [91]. Biomarkers that properly reflect the differences in the underlying

Table 2. Potential aging biomarkers

Justice *et al.* [30]	Inflammation	**IL6, CRP, TNF**, Eotxin, CMV antibody
	Nutrient sign	**IGF-1, Insulin, IGFBPs**,
	Cardio vascular	**NT-proBNP**, Fibrinogen, AGE/rAGE, tPA
	Stress response	**GDF15**, Isoprostane
	Others	**Cystatin C, DHEAS, Telomere length, Adiponectin**, TSH, **Leptin**
Cardoso *et al.* [93]	Inflammation	CXCL10, CX3CL1, IL-6, Pentraxin, sVCAM/sICAM, **Defensin**
	Mitochondria	**GDF15, FDNC5**, Vimentin, **APP**, LDH
	Ca homeostasis	**Regucalcin**, Calreticulin, S100B
	Fibrosis	PLAU, AGT, TGF-β, PAI-1, TGM2
	NMJ & Neurons	**BDNF**, Progranulin, sRAGE, **HMGB1**, C3/Caq, ST2, **Agrin**
	Cytoskeleton	α-KL, FGF23, FGF21, **Leptin, IGF1, Resistin, Adiponectin, Ghrelin, GH**
	Others	**miRNA**, AHCY, KRT18, GpnmB, Microparticle panel, Lactoferrin
Wagner *et al.* [94]	Physical and anthropometric	walking speed, chair stand, standing balance, grip strength, BMI, waist circumference, muscle mass,
	Blood-based	**IL-6, TNF-α, CRP, HbA1C**, glu, **Adipokines**, TSH, **Vit D, NT-proBNP**
	DNA based	**Telomere length**, DNA repair, DNA/chromosomal damage
	Novel	**Bilirubin**, AGEs, **MTs**, DNA methylation, **miRNA**
Guerville *et al.* [8]	Genomic instability:Micronucleus assay	Telomere attrition:**Telomere length**
	Epigenetic alterations:DNA methylation	Loss of proteostasis:**Clusterin**
	Nutrient sensing:**Sirtuin 1**	Mitochondrial dysfunction: **GDF-15**, Apelin
	Cellular Senescence:**P16INK4A**	Stem cell exhaustion:COP
	Inter. Communication: Inflammasomes, IMM-AGE score	

TSH: Thyroid hormones, AGEs: Advanced glycation end products, MTs: Metallothioneins, COP: Circulating osteogenic progenitors, NMJ: neuromuscular junction, Inter. Communication: intercellular communication, CMV: Cytomegalovirus, FNDC5: Fibronectin Type III Domain Containing 5, APP: Amyloid precursor protein, GH: Growth hormone, AHCY: Adenosylhomocysteinase, IGFBPs: insulin-like growth factor-binding protein, LDH: Lactate Dehydrogenase, BDNF: Brain-derived neurotrophic factor, HMGB1: High mobility group box 1, GDF-15: Growth and differentiation factor 15, TGM2: Transglutaminase 2, GpnmB: Glycoprotein Nmb, CRP: C-Reactive Protein, tPA: Tissue plasminogen activator.

age-adjusted biological aging processes are critical in the translation of information from invertebrate model organisms to vertebrate models in preclinical studies and subsequently in the clinical stage. In order for biomarkers to be useful in human clinical studies, they should be easily measured with minimal invasiveness, such as with blood, urine, or saliva samples, which could be collected throughout different investigation phases. Many blood-based and tissue-based biomarkers have been reported from aging research. The most comprehensive list of human aging biomarkers is available online in the Digital Ageing Atlas database (http://ageing-map.org) [92].

Four recently published panels of aging biomarkers are listed in Table 2. The TAME Biomarkers Workgroup selected 9 blood-based biomarkers as high-priority biomarkers and 11 as medium-priority biomarkers from 258 candidate molecules for aging and aging-related diseases. Those 9 high-priority biomarkers will be measured in a clinical trial involving 3,000 elderly subjects undergoing metformin intervention for 6 years [30]. There are still more markers available for consideration in small-scale human trials or animal studies. Cardoso *et al.* searched gene expression databases for aging and aging-related disease genes that can be detected in body fluid [93]. They identified 44 core panels of frailty biomarkers, consisting of 19 that were considered a high priority, 22 considered a medium priority, and 3 considered a low priority. Tissue distribution, concentration range in serum/plasma, and an overview of each of the biomarkers were clearly presented in tables in this study. Wagner *et al.* also selected a set of aging biomarkers, which included 9 blood-based markers, 3 DNA-based markers, and several relatively novel markers [94]. They considered some physical function and anthropometric markers, such as walking speed, chair stand, standing balance, grip strength, BMI, waist circumference, and muscle mass, as useful markers for aging evaluation. Both Cardoso *et al.* [93] and Wagner *et al.* [94] selected miRNAs as novel biomarkers. A growing number of studies have revealed that miRNAs are potential sensors of aging and cellular senescence [95–97], and several miRNAs, such as miR-21, miR-34a [98], and miR-146a [99], have been proposed to be useful biomarkers for aging and aging-related diseases. Guerville *et al.* selected one or two markers for each aging hallmark based on their association with mortality, age-related chronic diseases, frailty, and/or functional loss [8]. Their list included some unique markers that are hard to quantify in a timely fashion. Nevertheless, the micronucleus assay, a widely used genotoxicity test, was chosen for evaluation of genomic instability. Circulating osteogenic progenitor (COP) cells, a proposed surrogate marker of the mesenchymal stem cell population, have been used for evaluating stem cell exhaustion. As for altered intercellular communication (chronic inflammation), inflammasomes and the recently reported IMM-AGE score were chosen. There are also several other biomarker panels for aging, frailty, or sarcopenia research deserving our attention [100–102].

In Table 2, we summarized biomarkers for aging and aging-related diseases. All the chosen biomarkers are blood based, having consistent responses to aging and age-related disease conditions both in animal and clinical trials, and their costs of analysis are reasonably low, which makes them suitable in preclinical or clinical research of gerobiotics (biomarkers in boldface). However, it is still unclear whether the responses to specific gerobiotics can be observed in short-term interventions, e.g., 3 or 6 months. Any success for certain aging biomarkers responding to specific gerobiotics in preclinical experiments with short-term interventions would greatly facilitate further investigations in clinical studies using the same biomarkers.

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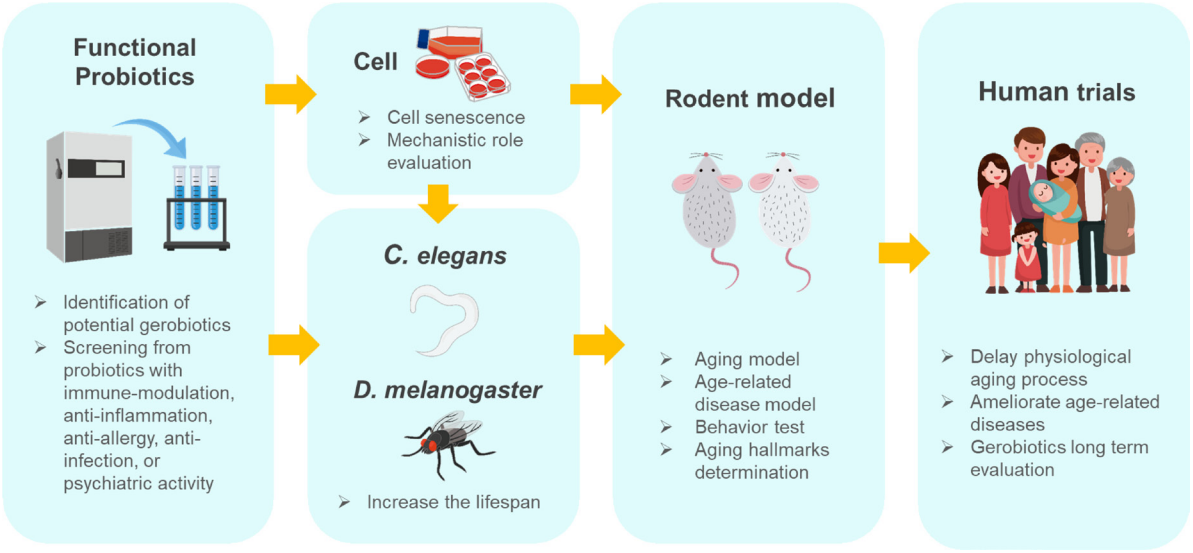

Fig. 2. The strategy for the identification of potential gerobiotics.

The first step is to screen potential gerobiotics from a functional probiotics bank and then to use cell and invertebrate models for high-throughput screening. Evaluation of aging biomarkers by multiple rodent models is a critical step in the process, since it can facilitate experimental designs for subsequent human trials.

HOW TO DEVELOP PROSPECTIVE GEROBIOTICS

Most probiotics, especially those with immune-modulatory, anti-inflammation, and anti-infection functions, are beneficial to improvement of quality of life for the elderly. To a large extent, they can all be generalized as gerobiotics. However, we reasoned that gerobiotics should be defined as those that are capable of attenuating the fundamental mechanisms of aging and reducing physiological aging processes, thereby expanding the health span of the host. In accordance with such a definition, we recommend a 3-step systematic approach for gerobiotic development. Firstly, evaluate the capabilities of probiotic candidates to extend lifespan in invertebrate animal models. Secondly, look for evidence of positive effects on multiple aging biomarkers in aging rodent models that can point out which fundamental processes are involved in the anti-aging effects of a candidate gerobiotic strain. Finally, similar positive trends in the same aging pathway should also be evaluated in well-designed human clinical studies. With such a systematic research approach, the biomarker data collected in preclinical or clinical studies can earn the trust of the general public and convey to them that long-term consumption of gerobiotics could bring benefits of extension of the healthy lifespan and reduction of aging-related chronic diseases.

Based on the abovementioned research approach, we propose detailed strategies for the development of potential gerobiotics (Fig. 2).

(1) In order to facilitate the screening process, it should start with those functional probiotic strains currently available that have shown immune-modulation, anti-inflammation, anti-allergy, anti-infection, or psychiatric activity.

(2) Some cell models developed for screening of small molecular geroprotectors can also be used for preliminary high-throughput screening from a large number of probiotic strains [103, 104].

(3) Potential gerobiotics could be screened from a functional probiotics bank, and then *in vitro* cell and invertebrate (*C. elegans* and *D. melanogaster*) models could be used for high-throughput screening.

(4) Rodents models should be positioned as the core of a screening pipeline so that every candidate is evaluated in multiple aging rodent models. Since probiotics are mainly delivered orally, distributed in the gastrointestinal tract, and excreted in feces, one might tend to focus only on those biomarkers that could be affected by the microbes resident in the gastrointestinal tract. However, we should not omit biomarkers that seem to be unrelated to the gastrointestinal system. Many potential biomarkers reported in published articles are considered, and a selection of them were chosen as examples and listed in Table 2. It is necessary to analyze as many biomarkers as possible in order to explore the entirety of all possible biological pathways that might be involved in the anti-aging activities of the studied strains. This will further facilitate the design of subsequent human trials. Not only aging rodent models but also other aging-related disease models, such as Parkinson's disease, Alzheimer disease, and frailty models, could be used to further validate the efficacy of the target strains. Even normal naïve rodent models can be used for analyzing changes of aging-related biomarkers, as in the case of *B. breve* B-3, which was described in Section 3.

(5) Ultimately, the anti-aging potential of gerobiotics will be determined by human trials. The observation of changes in certain physiological biomarkers, such as muscle mass, grip strength, bone mineral density, and gait velocity, would be very convincing. However, it is hard to observe these changes in interventions with gerobiotics in a relatively short term. Since it is impractical to observe effects on lifespan extension caused by gerobiotics in human studies, one should focus on the influence of the target strains on blood-based biological biomarkers selected based on animal studies.

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CONCLUSION

As a preventative intervention, probiotics have the benefit of low cost, ease of administration, high safety, and high user acceptance. Implementation of the geroscience-based approach will facilitate the development of anti-aging probiotics and the setting of effective marketing strategies. We therefore proposed the new term, gerobiotics, in order to highlight the importance of those anti-aging probiotic strains that have gone through sufficient preclinical or even clinical studies and had their positive effects validated with aging hallmarks. Several potential gerobiotics have been identified and evaluated with aging rodent models. Research on gerobiotics, although currently at an early stage, has the exciting potential to improve the health span of the elderly, which in turn will help resolve the silver tsunami problem. Those strains qualified as gerobiotics will demonstrate effects on prolongevity or improvements in multiple physiological characteristics of aging in *C. elegans, D. melanogaster*, or other aging rodent models. More importantly, it will delineate the biological pathway(s) that affects various aging biomarkers in preclinical or clinical studies. Gerobiotic candidates surely need to go through well-designed clinical trials to validate their anti-aging effects and the underlying fundamental pathways, even if only conducted in small-scale studies. As aging involves many intracellular signaling pathways, the joint action of several gerobiotics and other well-known geroprotectors aimed at different targets or processes may have additive or synergistic effects on life expectancy. Ultimately, the key to setting effective marketing strategies for gerobiotics is to accumulate solid preclinical and clinical evidence in order to convince business investors and to earn trust from the general public for long-term consumption. Postbiotics and para-probiotics derived from specific gerobiotics have been included in the definition of gerobiotics, since we anticipate their undeniable role in the extension of health span.

GLOSSARY

Geroscience: The Trans-NIH Geroscience Interest Group (GSIG), established in 2012, defined geroscience as an interdisciplinary field that seeks to understand the molecular and cellular mechanisms of aging, which is viewed as a major driver of common chronic conditions and diseases of the elderly. The aims of geroscience, as suggested by the GSIG, are to understand how aging triggers diseases and to exploit that knowledge to slow down the progression of age-related diseases and disabilities [19].

Geroscience hypothesis: The geroscience hypothesis suggests that any intervention targeting the basic biological mechanisms of aging will simultaneously delay the onset or even reverse the progression of multiple chronic diseases and functional decline and effectively expand the health span with greater efficiency [11].

Hallmarks of aging: In 2013, López-Otin *et al.* identified nine tentative hallmarks of aging, including genomic instability, epigenetic alterations, loss of proteostasis, telomere attrition, deregulated nutrient sensing, cellular senescence, stem cell exhaustion, mitochondrial dysfunction, and altered intercellular communication [7].

Aging biomarkers: Biomarkers of aging are molecular, cellular, and physiological parameters of the body that demonstrate reproducible quantitative or qualitative changes with age.

Geroprotector: Any intervention that aims to increase longevity or that reduces, delays, or impedes the onset of age-related pathologies by hampering aging-related processes, repairing damage, or modulating stress resistance [105].

Gerobiotics: Probiotic strains and their derived postbiotics and para-probiotics that are able to beneficially attenuate the fundamental mechanisms of aging, reduce physiological aging processes, and thereby expand the health span of the host.

Immunobiotics: Microbial strains that are able to beneficially regulate the immune system of the host [87].

Psychobiotics: Beneficial bacteria or support for such bacteria that influence bacteria–brain relationships [88, 106].

Postbiotics: Postbiotics refer to soluble factors secreted by live probiotics or released after bacterial lysis, such as enzymes, peptides, teichoic acids, muropeptides, polysaccharides, cell surface proteins, and organic acids [107, 108].

Para-probiotics: Para-probiotics are defined as nonviable microbial cells (intact or broken) or crude cell extracts which when administered in adequate amounts confer a benefit on the human or animal consumer [109].

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