Research progress on the mechanism of anti-aging evaluation system for Lactic acid bacteria

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Abstract: Lactic acid bacteria (LAB) is the general name of a class of bacteria that can ferment sugars to produce acid and gas. Lactobacillus has rich species diversity and geographical distribution, including at least 18 genera and more than 200 species. It is widely used in food, animal husbandry, medicine, and other fields. In recent years, due to LAB's excellent antioxidant and anti-aging properties, the research and development of corresponding functional products have become hot spots in various fields. Focusing on the excellent characteristics of antioxidation and anti-aging of LAB, this paper summarizes the evaluation system and analysis of effective active substances that can be used for screening anti-aging in order to provide the theoretical basis for screening functional LAB.

1 Instruction

Aging is a process of adaptation and regulation in the body, which is a gradual natural degradation of structure and function at all levels of the body. The main manifestations of aging are the following aspects: skin lesions, weakened cardiovascular system function, degradation of cardiovascular system-related organ function, the occurrence of hypertension, hyperlipidemia, weakened myocardial contractility, reduced cardiac output, and other neurological degradation[1]. Modern medicine has proven that some LAB is rich in vitamins, polyphenols, organic acids, polysaccharides, and other substances, which have unique advantages in antioxidants and anti-aging. Moreover, using LAB to ferment the raw material substrate can promote the precipitation and release of effective substances in the raw material. Therefore, constructing a screening system for LAB with high antioxidant and anti-aging properties has important theoretical significance and practical value for developing related functional products.

2 Evaluation of antioxidant capacity of LAB

The oxidative stress theory suggests that the human body's reactive oxygen species are in a dynamic balance. As age increases or disease damage, aging-related oxidative damage accumulates in the body, exceeding the body's clearance capacity. These accumulated reactive oxygen species exacerbate aging-related DNA damage and accelerate cell aging [2]. Therefore, the first step is eliminating excess reactive oxygen species and free radicals to delay aging. So to screen high anti-aging LAB strains, the first step is to screen antioxidant strains.

2.1 Metal ion chelation adsorption capacity

Some oxidation reactions in organisms require metal ions to undergo valence state changes to transfer electrons. Therefore, removing excess metal ions is particularly important for inhibiting oxidation reactions. Some organic acids and their derivatives, such as citric acid, vitamin C, can inhibit oxidation by chelating metal ions. Kai et al.[3] used salmonella-infected mice as experimental materials; it found that feeding LAB could reduce the content of metal ions in the intestinal mucosa of mice, thereby inhibiting Lipid peroxidation, thus improving the antioxidant level of the body. As essential to maintaining cell homeostasis, key rate-limiting enzymes, and energy metabolism, metal ions are significant for delaying aging by maintaining stable metal ion content.

2.2 Free radicals and the ability to remove reactive oxygen species.

Reactive oxygen species (ROS) refer to free and non-free radicals from oxygen sources, which have high chemical reactivity due to their unpaired electrons. The use of the two is similar in many cases. Evidence suggests that the aging process is accompanied by genomic damage, while oxidative stress significantly accelerates genetic damage [4]. Hongyu Wang et al. [5] research found that dozens of LAB, such as Lactiplantibacillus plantarum, Lactobacillus Rhamnose, Lactobacillus acidophilus, have reasonable DPPH clearance rates and Hydroxyl radical clearance ability. Among them, the DPPH clearance rate of Lactiplantibacillus plantarum exceeded 70%, reaching 71.37%; Genetic research has found that strengthening gene protection mechanisms can extend mammalian

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lifespan and eliminate excessive free radicals.

2.3 Reducing power

Reducing ability refers to the ability of a substance to lose electrons in a chemical reaction. Generally, the stronger the ability to lose electrons, the stronger its reducing ability (antioxidant ability). Therefore, reducing ability is also commonly used to evaluate antioxidant activity. Reducing agents can eliminate free radicals or reactive oxygen species by providing electrons. Ng et al. [6] fermented Taiwanese silver thread orchid using LAB, and the total antioxidant activity, reducing power, the scavenging effect of O2 • and peroxide of the fermented product were analyzed. After fermentation by LAB, the reduction ability at a wavelength of 700nm and its absorbance reached 0.3, while the ability to scavenge free radicals reached nearly 30%.

3 Evaluation of Aging Biological Models

After an initial screening of antioxidant capacity in the early stage, the high antioxidant strains obtained were applied to model organisms to evaluate the anti-aging performance of LAB more intuitively. As can be seen from the previous text, when the free radicals or reactive oxygen species in an organism accumulate excessively, they disrupt the balance in the body, causing oxidative stress and ultimately leading to aging. Overoxidation will lead to overexpression of some enzymes in the body and disorder of the body's functional structure, often accompanied by abnormalities of related enzymes and biochemical indicators [7].

3.1 Related aging Models

With the progress of science, researchers have further revealed the mechanisms of aging. Considering the experiment's comprehensiveness, economy, and focus, the selection of model animals is essential[8]. The commonly used animal aging models are summarized as table 1:

Table 1: Aging model and its advantages and disadvantages

Model name	Induction method	Disadvantage	Possible drug targets	Advantage
Elegans	Drug induced, genetically modified	Without heart, respiratory system	Regulatory transcription factor SKN-1 aging gene (daf-16 SKN-1) et al	Fast transmission; Low cost, easy to cultivate and observe, suitable for genetic analysis [9]
Mouse/Rat	Ozone, D-neneneba galactose	Long experimental cycle and high cost	Adjusting Wnt/ β-Catenin signaling pathway, inhibiting oxidative stress, etc	Mammalian, the corresponding characteristic expression is the closest to human body [10]
Zebrafish/bonito	Gene knockout and drug induction	Long experimental cycle; Low antibody selectivity	P53 signaling pathway mediates regulation of cell apoptosis and aging	Growth and development, tissue system, structural function， And the embryo development is transparent[11]
Drosophila	Gene knockout and drug induction	Big gap between Internal environment and nutrient absorption	Regulating Notch signaling pathway, p53 pathway, regulating cell cycle, etc	Fast reproduction, cheap price, and clear genetics; Having more mammalian homologous genes[12]
HSF and Immortal Keratinocyte	H_2O_2, UV	Inability to fully simulate biological life activities in vitro	Antioxidant stress, regulation of related genes such as SOD, MTH, and Rpn11	Strong amplification ability, easy to cultivate, and easy to operate [13]

3.2 Model application and detection indicators

3.2.1 Normal expression enzyme

Superoxide dismutase (SOD), Catalase (CAT), glutathione peroxidase (GSH-Px), and other essential components of the body's antioxidant system can be as important evaluation indicators for the anti-aging and antioxidant levels of lactic acid bacteria. SOD is one of physiology's most effective mechanisms for inactivating reactive oxygen species. Studies have shown that SOD weakens the decline in learning and memory induced by aging in model organisms by preventing oxidative stress-induced neurotoxicity[14]; CAT is the earliest discovered antioxidant enzyme that can catalyze the decomposition of hydrogen peroxide into water and oxygen. It is a critical enzyme in H_2O_2 and active nitrogen metabolism, and its expression and localization are significantly altered in tumors. However, the molecular mechanism regulating the expression of Catalase has yet to be fully clarified [15]; GSH-Px can reduce toxic peroxides to non-toxic hydroxyl compounds, which can reduce oxidative damage. LAB can enhance model organisms' resistance to heat and oxidative stress [16]. Li W et al. [17] evaluated the antioxidant activity of 10 LAB isolates through in vitro analysis and selected three highly active strains to study their probiotic function in Cryptococcus suis. Compared with the control group, the life span of Caenorhabditis elegans in the experimental group increased by 34.5%. It increases the exercise capacity of Caenorhabditis elegans, reduces the generation of age-related ROS and MDA damage, and promotes the production of SOD, CAT, and GSH-Px. The above experimental results indicate that LAB may be a potential probiotic strain for delaying aging and have particular research value.

3.2.2 Abnormal expression enzyme

The degree of accumulation of senescent cells in organisms can be measured through alternative indicators. Some preliminary studies directly adopt aging-related methods β-Galactosidase (SABG). With the development and progress of Proteomics, researchers have found that the number and type of proteins secreted by aging cells have changed significantly. The Matrix metalloproteinase (MMPs) family covers dozens of strictly regulated proteases. These enzymes can promote the degradation of extracellular matrix and play an essential role in tumor diffusion and migration; Elastase; Hyaluronidase mainly degrades hyaluronic acid, thereby; Causing skin moisture loss, leading to skin aging and various diseases; Overexpression of Tyrosinase leads to various skin lesions, including pigmentation, wrinkles, brown spots, and even Melanoma.[17,18,19]. Sol Lee et al. [20] researched the effects of cell-free supernatant (CNF) of Lactobacillus Formaticus BNR17 on anti-melanogenesis and oxidative stress of B16-F10 mouse Melanoma cells and HaCaT human keratinocytes were studied. They found that CNF treatment of BNR17 significantly inhibited CFS in B16-F10 cells and significantly reduced Tyrosinase activity. At the same time, CFS increases the expression of HO-1 and antioxidant-related genes as a potential anti-aging LAB.

3.2.3 Other testing indicators

In addition to the aforementioned enzymatic indicators, there is also increasing evidence that aging in organisms can be associated with many biochemical indicators, which explains the synergistic nature of the aging phenotype among different indicators[21]. For example, other markers in aging cells. 1. Factors related to cell cycle, such as p16, p53, p21, RB, Ki67, etc. Among them, the more common one is p53. The increased phosphorylation of p53 in organisms will activate cyclin- dependent kinase inhibitors (CDKI), ultimately leading to cell cycle arrest; The relevant factors secreted by aging cells, such as IL-1, IL-6, IL-8, VEGF, etc. Among them, IL-6 has a significant relationship with the occurrence of various diseases in clinical practice. An imbalance of IL-6 expression can cause various pathological changes; In addition, there is Lipofuscin that can be easily detected in Lysosome by an optical microscope. The abnormal indicators listed above can serve as indicators of aging. Because organisms are a complex and orderly system, different cellular tissues may experience differences in aging. Therefore, there is currently no genuinely absolute single marker of aging.

4 Current research deficiencies and prospects

This review briefly summarizes the anti-aging mechanism and application model of lactic acid bacteria. As an significant source of natural antioxidant and anti-aging substances, lactic acid bacteria have unique advantages in antioxidant and anti-aging, and have a wide range of applications in the food, cosmetics and pharmaceutical industries. The beneficial effects of lactic acid bacteria, intracellular extracts or fermentations on the body 's aging are manifested in the skin, including improving skin conditions and preventing skin diseases. In addition, due to the complexity of organisms, in order to deeply understand the complex biological process of aging, functional deficiency or acquisition of animal models is essential for research, which will provide causal evidence across the level of association analysis to support the meaning of relevant indicators in the aging process. China has unique advantages in the exploration of functional lactic acid bacteria resources. Therefore, it is of great significance to establish a scientific and reasonable functional lactic acid bacteria screening system for studying its mechanism of action and developing a strain library of lactic acid bacteria resources.

In the future, it is also necessary to use molecular biology, bioinformatics, analytical biology and other interdisciplinary disciplines to conduct research, which will be conducive to the systematic description and response of various characteristics of aging, but also conducive to the development of new interventions such as lactic acid bacteria. More importantly, it can explain the mechanism between aging and the process of aging. Therefore, the study of the mechanism of aging, revealing this precise causal network, and understanding the complex biological process of aging will be an exciting challenge in the future.

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