

Research on Probiotics and D-allulose

Kang Xu

School of Biological Engineering, Qilu University of Technology (Shandong Academy of Sciences), Jinan, Shandong, China

Abstract: D-allulose has attracted much attention because of its special taste and unique physiological functions, and it can be used as an ideal substitute for sucrose. At present, probiotics have been proven to have a variety of excellent physiological functions and are widely used in various fields.

Keywords: Probiotics; D-Allulose; Sucrose.

1. Probiotics

The term probiotics comes from the Greek [1]. According to the Food and Agriculture Organization of the United Nations (FAO) and the World Health Organization (WHO), probiotics are live strains of microorganisms that, when given in sufficient amounts, confer health benefits on the host, according to the International Association for Probiotics and Prebiotic Sciences The Scientific Association for Probiotics and Prebiotics (ISAPP) also follows this definition [2]. At present, probiotics mainly have the following categories: (1) lactic acid bacteria, including lactobacillus, bifidobacteri, streptococcus; (2) Non-lactic acid-producing bacteria, including Bacillus and Propionibacter; (3) non-pathogenic yeast, including yeast; (4) A class of bacilli or coccidiobacterium without spores and flagella. In recent years, a large number of studies have found that Lactobacillus acidophilus, Lactobacillus casei, Lactobacillus rhamnosus and Lactobacillus swiss in probiotics have been extensively studied in preventing human and animal diseases and have played a great role in human health.

2. The main function of probiotics

The alteration of the gut microbiota by probiotics is very prominent in the treatment of human and animal diseases, in addition to improving human gastrointestinal health and enhancing gastrointestinal function.

It also has (1) regulation of immune function: Studies have found that when probiotics colonize the mucous membrane, skin surface or between cells, they can generate a biological barrier to prevent the colonization of pathogenic microorganisms, and can stimulate the body's non-specific immune function, thereby regulating immune function [3].

(2) Lowering cholesterol: Probiotics can reduce cholesterol through mechanisms such as assimilation of cholesterol by growing cells, binding through fiber, and production of short-chain fatty acids by oligosaccharides [4].

(3) Alleviation of lactose intolerance: Recent studies have shown that fermented dairy products and probiotics can alter the metabolic activity of the colonic microbiota and may alleviate the symptoms of lactose intolerance [5].

(4) Removal of heavy metals and other functions: Probiotics reduce the absorption of heavy metals in the intestine by changing the expression of metal transporters and maintaining intestinal barrier function [6]. And it has a wide range of applications in food processing, pharmaceutical treatment, functional food, animal and plant microbial

preparations, etc.

3. D-allulose

D-allulose is an important rare sugar and a new low-calorie sweetener with the molecular formula $C_6H_{12}O_6$. D-allulose is found very little in nature, mainly in wheat, sugar cane, and beet molasses [7]. The study found that D-allulose is a white odorless powdered crystal compound with a molar mass of 180.156 g/mol, melting point of 96 °C, and 291 grams of dissolution in 100 g of water at room temperature, due to its unique physicochemical properties, D-allulose is an excellent substitute for sucrose in food, and its sweetness is about 70% of sucrose while the calories are almost 0 [8]. In 2011, D-allulose was approved by the Food and Drug Administration (FDA) as a safe food (GRAS) for use in a range of foods and dietary supplements [9].

4. D-allulose main function

D-allulose can not only be used as a low-calorie sweetener in beverages and dietary supplements, but also has a variety of physiological functions, compared with D-fructose and D-glucose, D-allulose has an important role in lowering blood sugar, anti-obesity, anti-atherosclerosis and so on.

(1) Hypoglycemic function: Studies have found that long-term use of D-allulose can induce the transfer of glucokinase from the nucleus to the cytoplasm of liver cells to maintain blood glucose levels in rats [10]. At the same time, D-allulose can promote the release of glucagon-like peptide (GLP)-1 in experimental mice, activate vagus afferent signals, reduce food intake and promote glucose tolerance in mice [11].

(2) Anti-obesity function. By studying the anti-obesity effect of D-allulose on adult rats fed a high-sucrose diet, it was found that 5% D-allulose significantly inhibited abdominal fat accumulation and weight gain in rats [12]. D-allulose supplementation resulted in significant reductions in weight gain and abdominal fat in rats without side effects compared with the same level of sucrose control [13].

(3) Anti-atherosclerotic function. Studies have found that high-density liprotein cholesterol (HDL-C) is directly related to the risk of atherosclerosis, and D-allulose can enhance the uptake of HDL-C by the liver by increasing the expression of class B type I scavenger receptors, reducing HDL-C levels, thereby preventing the development of atherosclerosis [14]. The discovery of D-allulose's anti-atherosclerotic function provides an option for subsequent drug studies of atherosclerotic diseases.

(4) Anti-oxidation function. D-allulose can be used as a

scavenger to clear the reactive oxygen species (ROS) produced after oral administration of di(2-ethylhexyl) phthalate in mice, and inhibit the production of ROS and malondialdehyde (MDA) in male rat testes, which has a preventive effect on testicular injury [15].

5. Probiotics and D-allulose in combination

At present, the research of probiotics and D-allulose mainly focuses on the biosynthesis of D-allulose, and the synergy between the two has been less studied, and studies have found that D-allulose may be beneficial to the growth and activity of certain in vitro probiotics, and D-allulose can inhibit acid production by certain lactic acid bacteria, but does not change their probiotic activity, which helps to develop new probiotic dairy products[16]. Synbiotic blends containing D-allulose were found to be effective in inhibiting diet-induced obese (DIO) by regulating lipid metabolism compared with the probiotic *Lactobacillus sakei* LS03, *Leuconostoc kimchii* GJ2, or D-allulose alone and its complications are more effective[17].

6. Biosynthesis of probiotics and D-allulose

In terms of D-allulose biosynthesis, in 1990, the Izumori team at Kagawa University in Japan discovered a strain of bacteria *Alcaligenes* sp.701B capable of converting D-tarotitol to D-allulose. The first reported biological reaction to produce D-allulose[18]. The novel D-allulose 3-epimerase (DPEase) of the *Bacillus* genus has high thermal stability and excessive epimerization ability. This biocatalyst has great potential for the large-scale production of D-allulose[19].

Natural DPEase is limited in industrial use due to its poor thermal stability, and by adopting a calculated disulfide bond design, the potential sites in the structure of *Clostridium* DPEase protein are selected to design new disulfide bonds can greatly improve its thermal stability and extend its half-life, increasing its industrial application potential [20]. The DPEase gene from treponemal ZAS-1 (Trpr-DPEase) was cloned and overexpressed and purified in *Escherichia coli* BL21 (DE3). Trpr-DPEase exhibits the best activity at pH 8.0 and 70 °C, is sensitive to temperature, has relative thermal stability below 50 °C, and shows maximum catalytic activity under a certain amount of metal Co²⁺ catalysis, with a conversion rate of 27.5% [21].

In summary, the combination of D-allulose and probiotics with clear probiotic functions, combined application of the two, can exert greater efficacy than the use of D-allulose and probiotics alone, and on this basis, targeted development of related products lays the foundation for subsequent research on the synergistic effect of D-allulose and probiotics.

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