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Review 综 述

益生菌冷冻干燥高活性保护机制研究进展

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摘　要：摄取足量益生菌有助于维持肠道微生物群落的稳态，对维持人体肠道健康具有重要意义。然而，在工业化应用中，益生菌抗逆能力较弱且对储存条件要求高，导致益生菌产品对运输和活性维持条件要求较高，这些产业需求对高活力益生菌的制备工艺提出了挑战。干燥处理常应用于保持益生菌活性和稳定性，其中冷冻干燥技术应用最广泛，但冻干过程中益生菌会受到各类环境压力的刺激，引起细胞损伤甚至死亡。因此，可以显著提高益生菌存活率的冻干保护剂成为目前益生菌工业应用的研究热点。本文从益生菌常用及新发现的冻干保护剂种类及其作用机制进行了系统归纳，对菌株冻干后细胞存活率的影响因素进行全面综述，并对冻干保护剂研究方向进行了展望，旨在为高活力益生菌冻干菌粉的研制提供理论支持。

关键词：益生菌；冷冻干燥；冻干保护剂；生理状态；脂肪酸调控

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Research progress in the mechanism of freeze-drying in protecting the high vitality of probiotics

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Abstract: Adequate intake of probiotics helps in maintaining the homeostasis of the gut microbiota, which is of great significance for human intestinal health. However, probiotics are vulnerable to stress and necessitate stringent storage conditions in industrial settings, which pose challenges to the vitality maintenance of probiotics in transportation and shelf-life. Therefore, it is essential to develop the preparation process capable of protecting the high vitality of probiotics. Drying beneficial for sustaining probiotic vitality is often used to maintain the strain stability. Among the drying methods, freeze-drying is widely used. However, during the freeze-drying process, probiotics encounter environmental stress conditions, which lead to cellular damage and even death. Therefore, the freeze-drying protectants that can significantly improve probiotic survival rate have become a research hotspot in the industrial application of probiotics. This article systematically summarizes both the commonly used and the newly discovered protectants for freeze-drying of probiotics and elucidates their mechanisms of action. It provides an overview of the factors affecting cell viability post freeze-drying while outlining the prospective research directions of freeze-drying protectants. This review is expected to furnish theoretical substantiation for the development of freeze-dried probiotics with high vitality.

Keywords: probiotics; freeze-drying; freeze-drying protectants; physiological state; regulation of fatty acids

联合国粮食及农业组织将益生菌(probiotics)定义为“活的微生物，当其摄入足够量时，能为宿主带来健康益处”[1]。摄入足量益生菌有助于调节和改善宿主肠道微生物群的稳态，对人体健康的潜在作用被广泛证实，已在食品、生物医药等多个领域得到广泛应用。Ejtahed 等[2]总结了益生菌、益生元和其他膳食成分(如多酚)通过调节肠道微生物群进而控制食欲，减少肥胖症的发生；Wang 等[3]的研究表明复合益生菌能够通过激活 Toll 样受体(Toll-like receptors, TLR)信号通路平衡 M1/M2 极化，对调节宿主血糖水平和保护黏膜屏障功能发挥积极作用；Soemarie 等[4]总结了不同发酵食品中有不同种类的益生菌，同时也总结了乳杆菌在发酵乳、巧克力等食品中的应用。我们研究团队已建成了相关的健康功能微生物菌种资源库(库容>2 万株)及基因数据库并进行功能挖掘，目前已证明益生菌能够通过调节肠道微生态从而在降

糖、降脂等方面产生显著效果[5-7]，Yang 等[8]发现副干酪乳杆菌 201 能减轻高胆固醇血症大鼠的症状；Jiang 等[9]筛选出长双歧杆菌 070103 可以改善高糖、高脂饮食诱导的小鼠的葡萄糖和脂质代谢紊乱。

然而，益生菌对宿主的影响作用存在量-效关系，只有摄入足够量的益生菌才能提升宿主健康。国际乳制品联合会建议，在益生菌食品中，活菌含量应达到 10^6–10^7 CFU/mL 才能确保其益生功能[10]。在益生菌的工业化生产过程中，由于产品运输、储存等环节的环境条件均不利于微生物生存，导致益生菌活性难以维持。研究证实，干燥是保持益生菌活力的重要因素，而将益生菌制成菌粉是目前维持益生菌稳定性最有效的方法[11-12]。目前常见的干燥方法包括喷雾干燥、冷冻干燥、真空干燥和流化床干燥[13-14]。虽然喷雾干燥成本较低、所制备的菌粉颗粒更细腻，但是目前多数研究结果均指出，冷冻干燥对于益生菌活力的维持优于喷雾干燥[15]，是目前高活性益生菌制备的首选方法[13-14]。

冷冻干燥(冻干)是一种基于冰晶升华的原理，利用物质升华脱水的特性干燥物质的技术。在冻干过程中，益生菌细胞将面临机械应力、逆境胁迫等多种压力，导致细胞膜的完整性、流动性和敏感蛋白结构受损，造成生理功能损伤甚至引发菌体死亡。在冻干过程中细胞损伤主要归因于细胞内冰晶形成、大分子低温变性以及细胞内部溶质丢失导致的高渗透压[12,16]。因此，解析益生菌在冻干环境的损伤机制，并研发针对性的保护技术，有利于高效提高冻干菌株的存活率。

大量研究表明，通过添加合适的外源保护剂、调整冻干流程，可以有效提高益生菌菌株的存活率和细胞活力[14]。Cheng 等[17]通过对比植物乳杆菌和发酵乳杆菌在冻干过程中添加与不添加保护剂后菌株的存活率，证实保护剂能显著提高多种菌株在冻干过程中的存活率。然而，不同的冻干保护剂对不同菌株的保护作用存在差异，这表明冻干保护剂的作用具有菌株特异性。因此，根据菌株的生理特性研制保护剂方案，对于制备高活性益生菌具有重要意义。此外，冻干前菌株的生理状态也与冻干后细胞活性密切相关，Hernández 等[18]探究了发酵培养基 pH 和发酵温度对罗伊氏乳杆菌 DSM 17938 抗逆性的影响，发现在高 pH 发酵的菌株抗逆性更强，冻干后细胞的存活率更高。因此，本文将系统综述益生菌在冷冻干燥中所使用的保护剂种类及其作用机制，同时归纳了目前针对冻干前菌株生理状态对存活率影响的研究现状，综述冷冻干燥技术在益生菌研究领域的最新进展。

1 冻干前菌株生理状态对存活率的影响

研究表明，益生菌在干燥过程中的生理状况是其实现高生存率的关键因素[19]。研究指出，以下 3 种方式均对冷冻干燥后益生菌的细胞活性产生影响：(1) 调节不饱和脂肪酸与饱和脂肪酸比例以保持细胞膜流动性；(2) 上调应激蛋白的活性和合成以保护细胞结构；(3) 促进抗氧化物防御物质的积累以提高抗逆性等[18]。总体而言，在冻干前使菌株进入逆境胁迫、诱导生物被膜(biofilm)形成以及增加菌株细胞膜不饱和脂肪酸含量的生理状态，均有利于冻干后益生菌的生存率。

1.1 逆境胁迫

逆境胁迫是指利用亚致死环境(如酸应激、热应激等)刺激菌株，使其启动自身的保护反应，从而提高菌株抗逆性，维持其在冻干后的存活率[14]。Nguyen 等[20]利用温度、pH 和二氧

化碳对 3 株不同的益生菌进行逆境胁迫后再进行冷冻干燥，证实逆境胁迫后菌株会产生更多的胞外多糖(exopolysaccharides, EPS)，可在冻干过程中显著提升细胞的存活率。Nguyen 等[21]发现，逆境胁迫会导致菌株合成具有自我包裹与保护作用的 EPS，与冻干保护剂形成双层微封装结构，从而提升菌株冻干后的活力。除了产生 EPS 外，逆境胁迫还可以诱导菌体内特定蛋白(如热休克蛋白)的瞬时表达，而这些蛋白的表达有利于增强菌株在冻干环境的耐受力，进而提升其冻干后的活力。Shin 等[19]通过 52 ℃加热 15 min 的热胁迫刺激粪肠球菌 HL7 发现，热刺激不仅明显提升了菌株冻干后的存活率，还会增强菌株在酸、碱环境下的耐受性。Zhen 等[22]同样利用热应激处理嗜酸乳杆菌 ATCC 4356，发现经过处理后，菌株提高了胞内葡萄糖的利用率、合成更高产量的乳酸和多糖，从而提高了冻干存活率。因此，在益生菌冻干前给予适当的逆境胁迫预处理，可以最大程度地提高菌株在冻干过程中的存活率。深入研究菌株逆境胁迫与冻干保护之间的分子关联机制，将对利用逆境胁迫策略提升冻干细胞存活率提供理论指导。

1.2 生物被膜形成

生物被膜是指附着于特定物质表面的微生物聚集体，其通过释放胞外聚合物来增强菌株的抗逆性[23]。除了优化冻干保护剂组分外，目前研究指出，通过优化益生菌的培养基成分或者调整其培养条件，可以增加菌株生物被膜的形成量，显著提升其在冷冻干燥后的存活率[24]。E 等[24]研究发现通过增加培养基中的钾离子浓度，提升益生菌 LuxS/自诱导剂-2 (autoinducer-2, AI-2)群体感应系统中 *luxS* 基因的表达，从而增加下游信号 AI-2 的合成，可以上调 *cysE* 基因，促进生物被膜的形成，这些生物被膜在冻干过程中明显提高了菌株的存活率(图 1)。Salman 等[31]总结了群体感应系统参与调节益生菌的生物被膜形成，并阐述了益生菌生物被膜的形成可以提高菌株对不良环境的应激抗性。类似地，孙瑞胤[32]报道，在培养基中添加钙离子以及在不同阶段给予菌株盐胁迫，植物乳杆菌 LIP-1 菌体变短、生物被膜形成量增加，进而使菌株冻干后的存活率增长了 69.63%。另一方面，Gaucher 等[33]则通过调整费氏丙酸杆菌 CIRM-BIA129 的培养基 C/N 组成，增加细胞间渗透保护剂的积累量，从而刺激生物被膜的形成，同时提升了菌株的耐热性和冻干存活率。此外，应用非生物物质刺激生物被膜形成也是提升益生菌抗逆性的重要手段。Liu 等[23]研究发现，采用粒径为 80–120 μm 的葡萄籽粉(grape seed flour, GSF)可刺激双歧杆菌的生物被膜形成，在培养 60 h 后，不含 GSF 的培养基中几乎无活细胞，而含有 GSF 的培养基中活细胞数超过 10^6 CFU/g，表明生物被膜可以提高菌株在逆性环境中的抗性。

1.3 细胞膜不饱和脂肪酸调控

益生菌在低温和渗透压力下的生存状况与其细胞膜脂质组成有关[34]。如图 2 所示，当益生菌的细胞暴露于低温等压力环境时，细胞外部介质会发生浓缩、膜硬化和细胞脱水，进而损害细胞膜的完整结构，而增加细胞膜不饱和脂肪酸的浓度可以增强其流动性，减轻冻干损伤[35]。陈境[36]通过调节培养基 pH 值促进植物乳杆菌细胞膜环丙烷脂肪酸的合成，发现在 pH 6.8 的低酸环境中，菌株不饱和脂肪酸的合成量增加，这些不饱和脂肪酸在冻干过程中保护了细胞内关键酶的活性，进而提升了菌体活力。E 等[34]通过增加培养基中钾离子的浓度，上调植物乳杆菌 LIP-1 的脂肪酸代谢基因表达，增加培养过程中菌株不饱和脂肪酸的合成，提高细胞膜的流动性，从而使菌株具有更强的抗冻干

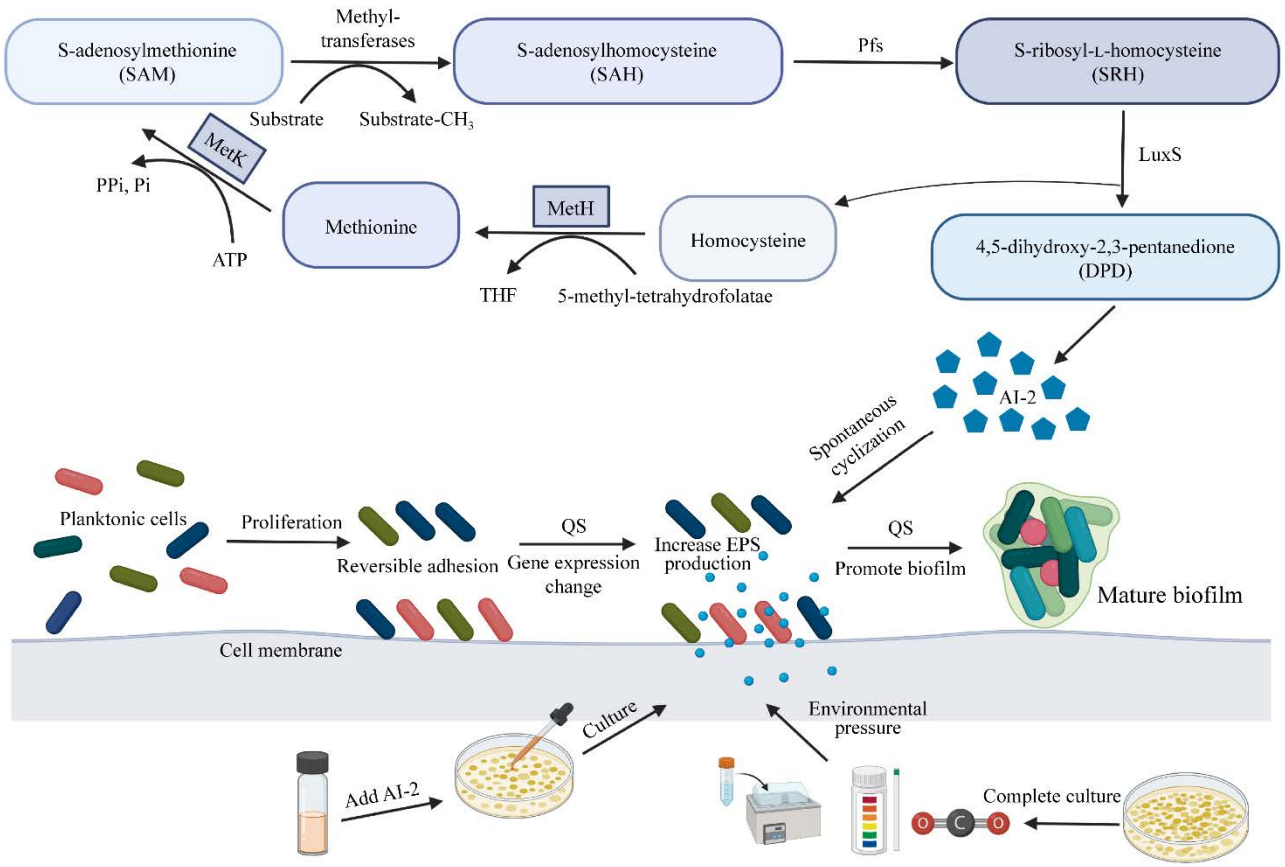

图 1　基于 LuxS/AI-2 的群体感应系统调控生物被膜形成[25-30]

Figure 1　The regulation mechanism of biofilm formation by LuxS/AI-2 quorum sensing[25-30].

能力。Wang 等[37]发现在植物乳杆菌培养基中加入十八碳烯酸(C18:1)后，可以有效维持细胞膜的完整性和流动性，提高胞内敏感酶的活性，从而增加菌株冻干后的存活率。但是，外源添加脂肪酸的含量并非越多越好，何宗柏等[38]发现低质量浓度(≤0.2 g/L)的油酸($C_{18:1\omega9c}$)可以促进棕榈酸($C_{16:0}$)转化为环丙烷脂肪酸($C_{19cyc11}$)，同时诱导细胞内饱和脂肪酸向不饱和脂肪酸转化，使植物乳杆菌 LIP-1 冻干存活率增加 8.38%，然而，高质量浓度的油酸会抑制菌株生长。因此，探索合适的外源脂肪酸添加量对于提高菌株的冻干活性具有重要意义。

近年来，关于冻干存活率的研究逐渐趋向增加益生菌的生物被膜含量和改变其冻干前的生理状态，但是处理手段较为复杂，以及如何降低工业化应用的生产成本问题亟待解决。

2　冻干保护剂的种类及作用机制

在冷冻干燥过程中，极低温和干燥脱水等极端环境对细胞活性产生不利影响，从而降低菌株的存活率。在冻干前添加相应的保护剂是减轻菌株在环境压力下造成细胞损害的策略[39]。研究发现，冻干保护剂在提高细胞存活率方面具有重要作用，其主要作用是扩大未冷冻部分、防止冰晶挤压造成细胞损伤[39]。常见的保护剂类型包括糖类、蛋白质类、高分子物质、氨基酸类、醇类和抗氧化类等，而这些保护剂的作用机制也有所不同(图 3B 和表 1)。

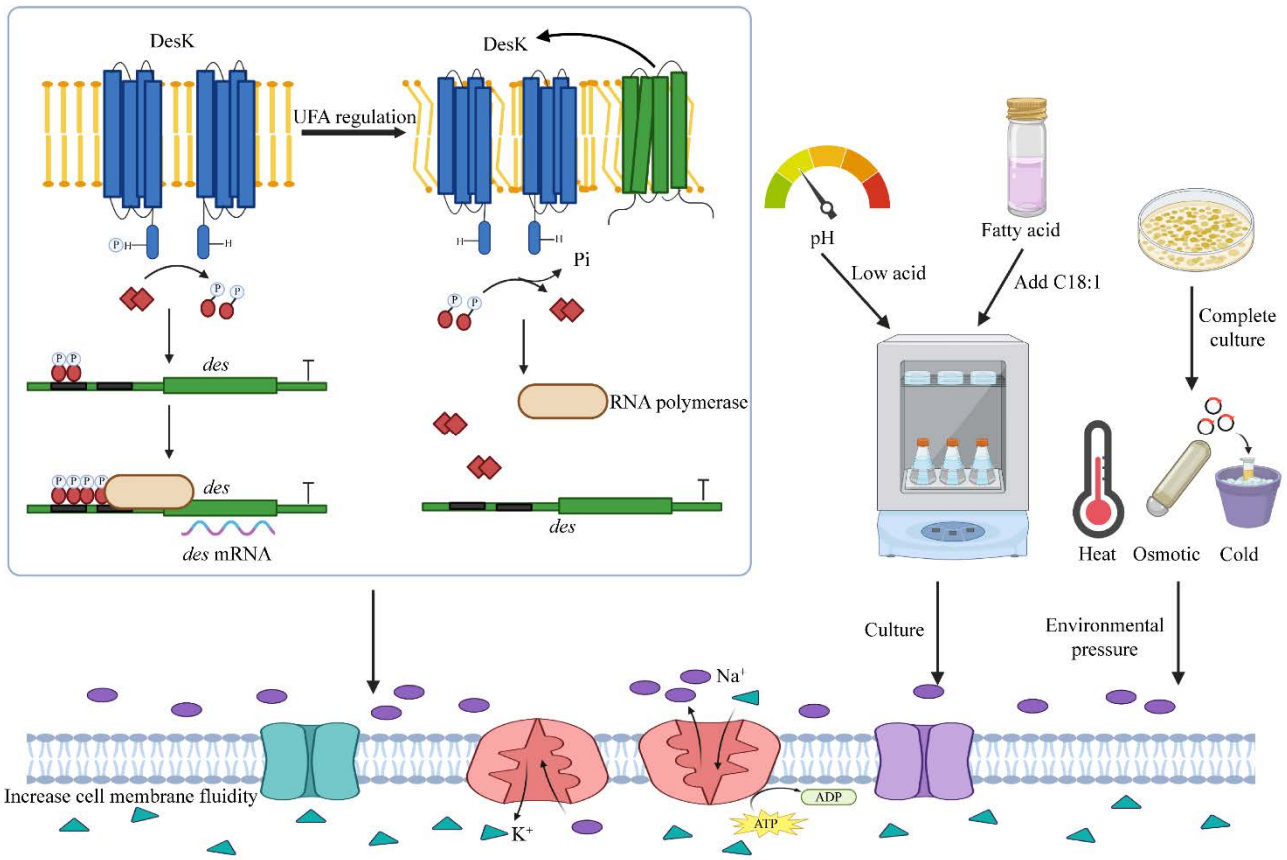

图 2　脂肪酸和环境压力对细胞膜流动性的影响

Figure 2　The impact of fatty acid and stress environment on cell membrane fluidity.

2.1　不同材料的冻干保护剂作用特性

益生菌的冻干保护剂通常可分为 3 种类型：(1) 稳定剂，其作用是与蛋白质和膜磷脂的极性头基形成氢键，并在解吸未冷冻水的过程中充当水的替代物；(2) 高分子量多糖或聚合物，其作用为增加溶液的玻璃化转变温度；(3) 抗氧化剂，其作用是限制冷冻干燥产品储存后的氧化反应[49]。研究发现，冻干保护剂的效果存在菌株特异性，因此在选择保护剂时需依据菌株自身的特性进行筛选。Heravi 等[45]研究了 4 种不同组分的保护剂对 3 株乳杆菌的冷冻干燥保护效果，其中含有乳清和麦芽糊精的保护剂对唾液乳杆菌 20687 保护效果最佳，含有蛋白胨和蔗糖的保护剂则对植物乳杆菌 NRRLB-14768 的保护作用更强，而鼠李糖乳杆菌 GG 在含乳清和蔗糖的保护剂中达到最高的冻干存活率。因此，在选择冻干保护剂时需考虑菌株本身生理特性的差异。在此基础上，复配保护剂比单一保护剂的保护效果更佳，因为不同材料能在不同位点协同发挥作用，从而最大程度降低外界环境对益生菌的影响[50]。Wang 等[51]分别应用蔗糖、甘露醇、麦芽糖糊精和脱脂奶粉等成分作为益生酵母 MJ1 的冻干保护剂，其存活率均低于 50%，而复配这些组分，则可将细胞存活率提升至 90.21%±1.04%。因此，通过复配不同的材料，冻干保护剂可以发挥最大作用。

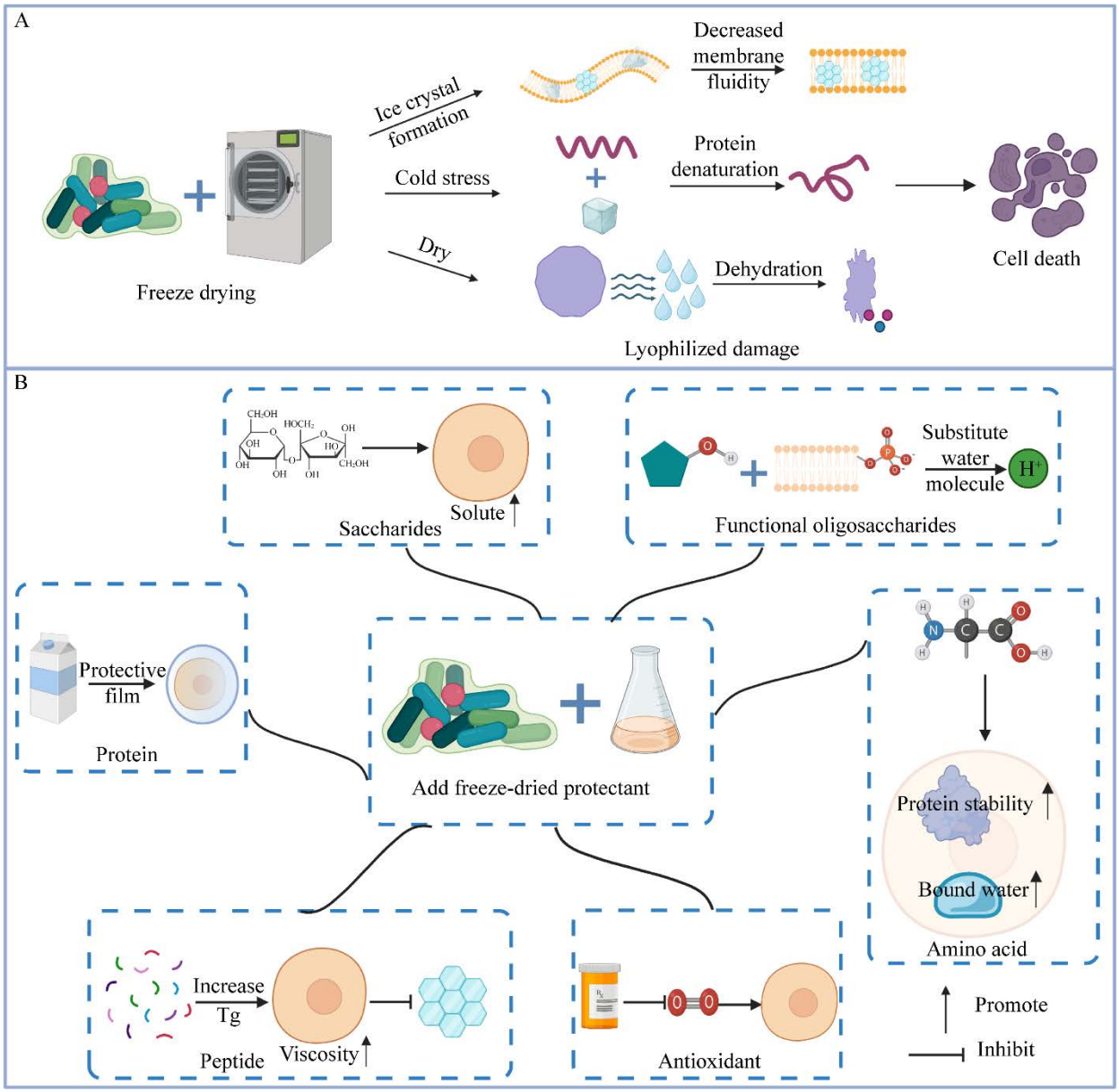

图 3　不同种类冻干保护剂的作用机制

Figure 3　The mechanism of different kinds of lyophilized protective agents. A: The damage mechanism of freeze-drying. B: The mechanism of lyophilized protective agents. Tg: Glass transition temperature.

2.2　糖类

糖类是益生菌冻干保护剂中重要组分，根据其结构和分子量，糖类又可分为单糖和多糖。糖类保护菌株的作用是通过细胞壁进入细胞，增加细胞内溶质的浓度，从而维持细胞内外渗透压平衡[51]。李兵兵等[52]以植物乳杆菌 Lp-115 为实验菌探索了银耳多糖的冻干保藏作用，发现银耳多糖在冻干过程中可以降低菌粉表面的孔隙率，抑制细胞膜受损，并保护菌体关键酶的活性。我们在筛选植物乳杆菌 LP1Z 糖类冻

表 1　冻干保护剂在不同菌株中的应用

Table 1　Application of lyophilized protectants in different strains

Strains	Lyophilized protectants	Mechanisms	References
Bifidobacterium brevis CCFM683	Sorbitol, raffinose, collagen	Sorbitol improved osmotic stress, stabilized protein structure and resisted oxidation; raffinose accumulated and interacted with water molecules in cells; collagen provided a protective layer and formed a porous structure for cells	[40]
Lactobacillus plantarum L1	10% skim milk, 13% sucrose, 2% sorbitol, 0.8% tyrosine	Skim milk stabilized cell membrane components and provided a protective layer of proteins; the hydroxyl group of saccharides formed hydrogen bonded with the phosphate group of a lipid; amino and carboxyl groups of amino acids interacted with water molecules to form hydrogen bonds, increased the viscosity of the solution and reduced cell damage	[17]
Lactobacillus plantarum TISTR 2075	10% rice protein, 5% fructooligosaccharide	The amino group of rice protein interacted with the carboxyl group of bacterial protein to maintain the cellular protein structure; the interaction between fructooligosaccharide and cell membrane reduced the mechanical damage of cell membrane	[41]
Lactococcus lactis GH1	10% galactose, 10% trehalose	Saccharide formed a glassy structure that protected intracellular proteins and displaced water between lipid head groups to prevent cell membrane drying and rehydration	[42]
Lactobacillus curvatus N19	20% skim milk, 3.57% lactose, 10% sucrose	Saccharide and protein worked together to reduce osmotic shock, cell membrane damage, macromolecular deformation, membrane lipid inactivation, and structural changes of sensitive proteins in biological systems	[43]
Lactobacillus acidophilus LA-5	15% polymerized whey protein	Polymerized whey protein formed a dense protective film	[44]
Lacticaseibacillus rhamnosus GG	0.5% whey, 27% sucrose, 12% skim milk	Sucrose interacted with water molecules to form hydrogen bonds; skim milk reduced mechanical damage to bacteria under environmental stress	[45]
Ligilactobacillus salivarius 20687	0.5% whey, 1% maltodextrin, 12% skim milk	Maltodextrin and proteins formed a protective layer on the surface of bacteria to reduce the damage of environmental stress to cells	[45]
Lactobacillus reuteri F-9-35	19% maltodextrin, 12% straw mushroom polysaccharide extract, 10% fish collagen peptide	Polysaccharide and peptide formed a dense protective layer on the surface of the bacteria and increased the nutrient diversity of the bacteria powder	[46]
Lactobacillus paracasei JCM8130	10% carnosine	Carnosine served as antioxidant and provided glass phase transition properties similar to disaccharides	[47]
Bifidobacterium animalis ssp. *lactis* MG741	1% glutathione	Glutathione protected key enzyme activity and prevented cell membrane fatty acid peroxidation	[48]

干保护剂时，发现抗性糊精对其冻干保护作用显著。此外，糖类保护剂除了维持菌株在冻干过程中的功能结构外，还能作为碳源在后续发酵时为菌株提供生长所需营养成分。Jawan 等[42]使用 10%半乳糖和海藻糖作为乳酸乳球菌 Gh1 的冻干保护剂，获得最高细胞活力和储存稳定性，并推断使用此组合保护剂不仅能在冻干过程中保护细胞，还能被微生物生长利用，促进微生物的生长，而这与生长培养基组分相关。

近年来，功能性低聚糖成为了冻干保护剂的一个热点新材料。由于其结构中含有 2−10 个相同或不同的单糖，而每个单糖均含有自由羟基，与甘油类似，功能性低聚糖的羟基可部分取代水分子与菌体细胞膜磷脂中的磷酸基团(或者与菌体蛋白质极性基团)形成氢键，使菌体中的大分子物质在缺水状态下仍能保持原有的功能结构[53]。张菊等[53]利用功能性低聚糖(如低聚木糖、低聚果糖、水苏糖和低聚异麦芽糖等)作冻干保护剂，发现低聚木糖对植物乳杆菌的冻干保护率可达 80%。这些功能性低聚糖不仅能提升益生菌的活性，还能促进宿主健康状态，具有良好的应用前景。

2.3 蛋白质类

蛋白质类物质如植物蛋白和脱脂乳也是冻干保护剂中的重要组分，这类物质可以为益生菌提供保护性外膜，减少细胞损伤[51]。在各类蛋白质保护剂中，脱脂乳因其能在菌株外层形成保护层、缓冲应激损伤且价格低廉的特性，目前被广泛用于工业生产中[54]。徐颖等[54]利用脱脂乳作为基础保护剂探究富硒鼠李糖乳杆菌的冻干保护方案，发现在脱脂乳基础上添加海藻糖和谷氨酸钠能使菌株的冻干存活率提升至 89.86%。辛明等[55]应用脱脂乳作为基础配方优化植物乳杆菌 L5 和 L12 的冻干保护剂，发现以其为基础联用海藻糖和硫酸铵，可使植物乳杆菌 L5 冻干后的存活率提升至 82.55%，而植物乳杆菌 L12 的存活率提升至 83.66%。

进一步研究发现，不同蛋白质类物质在冻干过程对菌株的保护作用也存在差异。Xie 等[56]证实乳清蛋白水解物能缓解冻干过程中细胞的渗透休克，提高鼠李糖乳杆菌 CICC 22152 冻干存活率；Kim 等[57]以食窦魏斯氏菌 JW15 为研究对象，解析了脱脂乳、大豆粉和酵母粉这 3 种蛋白质对益生菌的保护作用，其中，大豆粉和酵母粉能明显增强菌株的抗冷冻干燥能力，而脱脂乳和大豆粉组合则能增强菌株对酸性溶液的耐受性；Bodzen 等[58]发现胶束酪蛋白可降低化学降解反应所需水分，降低菌粉中的水分活度，延长植物乳杆菌 CNCMI-4459 冻干粉储存稳定性。Chen 等[59]制备的重组抗冻蛋白可以在嗜热链球菌的胞外荚膜多糖、肽聚糖和冰晶之间相互作用，调节冰晶的分子结构、减少外膜损伤、抑制细胞凋亡并增加细胞内代谢活性，提高嗜热链球菌在冷冻应激下的存活率。源于生物中的重组抗冻蛋白对益生菌的冻干保护效果更好，有望将其作为新型的冻干保护剂替代传统的冻干保护剂应用于工业生产中，但是，重组抗冻蛋白的安全性及稳定性仍需进一步研究。

然而，目前被用作冻干保护剂的蛋白质类物质仍相对较少，而拓展新型蛋白质类冻干保护剂以及安全性评价将为益生菌的工业化生产提供更多选择。

2.4 肽类物质

肽类是介于大分子蛋白质和小分子氨基酸之间的活性物质，目前已逐渐被开发为新型冻干保护剂[47]。益生菌保护剂的玻璃化转变是指其在冷冻干燥过程中形成玻璃状(极高黏度)基质，继而固定菌株细胞、抑制冰晶形成，使菌

株免受脱水及冷冻的影响[16]。研究发现，肽类物质可以提高冷冻干燥物料玻璃化转变温度，减少益生菌冷冻损伤，提高菌株的活性[47]。Mikajiri 等[47]发现肌肽(β-丙氨酸-L-组氨酸)具有与糖相似的提高玻璃化温度的特性，可保护罗伊氏乳杆菌、嗜酸乳杆菌、副干酪乳杆菌等多种益生菌在冻干后的活性，同时维持这些菌株在储存期间的稳定性。

除了提高玻璃化转变温度外，肽类物质还可通过多种机制在冻干过程中保护益生菌的活性。Kwon 等[60]发现丝素蛋白作为一种具有 β-折叠结构的天然肽类聚合物，其结构中的氢键可在冻干过程中提供疏水相互作用，保护益生菌细胞免受冷冻损伤。Kim 等[61]发现胶原蛋白肽能有效改善益生菌冻干过程中所受的损伤，并提高菌株的胃肠道耐受性及热稳定性。尽管肽类物质在益生菌食品工业化中表现出巨大的应用潜力，但其高昂的价格及有待确证的安全性使其在推广应用中受到了一定的限制，开发兼具冻干保护剂和益生元功能食品级多肽类物质是未来研究的热点领域。

2.5 其他

除上述物质外，近期研究发现一些新型材料可被用作冻干保护剂，如高分子物质麦芽糊精等[51]。研究指出，麦芽糊精可以增加应变细胞悬液的黏度，同时提高冷冻干燥物料的玻璃化转变温度，使整个冻干体系难以结晶，且制得的粉末蓬松、易于复水，因此有更好的菌株活力及产品形貌[51]。除此之外，氨基酸物质如L-谷氨酸钠等可以穿透细胞内部，与微生物细胞内的蛋白质氨基结合，在冻干过程中稳定蛋白质结构，维持益生菌蛋白的功能；同时，这些氨基酸可以保留细胞内的结合水，减少干燥过程中细胞内的过度失水[51]。另外，一些醇类物质如山梨醇、甘露醇等也展现了良好的冻干保护功效，这些醇类物质通过其羟基与益生菌膜蛋白的极性基团或膜磷脂的磷酸基团结合，在冻干过程中稳定了菌体内蛋白质的结构，并保护细胞膜的完整性。而抗氧化剂如抗坏血酸，也因其具有防止有害胺和羰基对益生菌的氧化损伤作用，常被用于益生菌的冻干保藏中[51]。

除了在冻干过程中保护菌体免受损害外，保护剂还会在再水化过程中为益生菌提供营养，维持菌体细胞的活性。Arellano 等[62]发现一种基于赖氨酸的混合物可通过调节再水化过程中菌体细胞的 zeta 电位，维持细胞的活力，并提升细菌在模拟胃肠液中的耐受性。Arellano-Ayala 等[63]证实利用碳水化合物、蛋白质和含氮分子材料作为冻干保护剂有利于维持益生菌再水化后的细胞 zeta 电位，增加菌粉的蛋白黏附性，提高菌株的再水化活力。而基于上述发现，Arellano-Ayala 等[63]提出，冻干保护剂的选择除了需要评估其在冻干过程的保护效能外，还需考察其在再水化过程中的功效；同时，保护剂与菌泥的混合比例、混合后的平衡时间也可能引起菌体细胞渗透压的改变，应纳入保护剂制备的评估体系内[64]。

近年来，关于提高冻干保护作用的研究主要集中于保护剂新物质的开发，除此之外，冷冻干燥工艺的程序设计、保护剂与冻干工艺之间的关联等仍较为缺乏，而对于冻干保护体系的整体研发对益生菌工业化生产具有重要的意义。

3 展望

传统益生菌冻干保护的研究主要聚焦于三大方向：冻干保护机制的解析、不同冻干剂复配方式的探讨以及基于菌株特异性的保护剂筛选。近年来，基于冻干保护剂与培养基组成

成分的关联也成为了该领域研究的新方向，而创制新型冻干保护剂材料也是该领域的热点问题，但其在生产中的性价比以及潜在的安全性问题在一定程度上限制了新材料的应用。除了保护剂材料的选择外，明确现有冻干保护剂与冷冻干燥工艺之间关联也是提升菌株活力研究的重要方面。通过环境压力形成应激反应、优化菌株发酵培养基以及调整菌株发酵条件，有利于提升菌株的生理状态应对冻干损伤。然而，目前相关研究主要集中在热应激和酸应激方面，而冷应激、饥饿应激、氧化应激等其他环境压力是否可以促进菌株的抗冻能力则有待进一步研究。

综上所述，本文系统阐述了益生菌冷冻干燥中的保护策略，总结了益生菌冻干保护剂的组分及作用机制、冻干前菌株生理状态对存活率的影响及其机理。在此基础上，研究者们在未来工作中可从下述 3 个方面推动冷冻干燥技术在益生菌产业化中的应用：(1) 建立新型材料作为冻干保护剂的安全评价体系；(2) 探索冻干保护剂与冷冻干燥工艺的关联；(3) 揭示不同环境压力下菌株产生的自身保护机制，以提供提高冻干存活率的理论依据。

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