

益生菌抗冻干损伤方法研究进展

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摘要 近年来,益生菌产品在食品、医疗保健等行业中应用广泛,然而在其工业化生产、运输和贮藏过程中,保持菌株活性面临挑战。目前,企业与研究机构多采用干燥的方法来维持益生菌的活性,其中,真空冷冻干燥技术因能够最大程度地保持菌株活性,能耗低,工艺简单而成为主流选择。然而,真空冷冻干燥技术仍会对益生菌的细胞膜、酶、DNA 等造成损伤,降低其在应用过程中的活性。本文总结益生菌抗冻干损伤方法的最新研究进展,即通过调控培养基组分与培养条件,胁迫预处理,优化离心条件,添加冻干保护剂,优化冷冻干燥工艺,来增强菌株的抗冻干能力,降低冻干损伤,旨在为制备高活性的益生菌产品提供理论参考。

关键词 益生菌; 冷冻干燥; 损伤; 方法; 活性

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益生菌是具有活性,非致病性,能够耐受胃肠道条件,并可在结肠中定植,发挥有益健康效应的微生物,当给予适当剂量时,有效活菌数最低可达 10^6 CFU/g^[1]。研究表明,益生菌可以延缓衰老^[2],抗肿瘤^[3],增强肠道屏障功能,减少炎症,缓解慢性腹泻^[4],降低牙龈炎症^[5],改善口腔健康^[6],并在癌症的治疗和预后中发挥重要作用^[7]。

近年来,益生菌产品因具有诸多益生功能而深受消费者的喜爱。为方便贮存、运输和生产高活性的益生菌产品,研究人员、企业及生产厂家多采用干燥方法制备菌粉。目前,干燥方法主要包括真空冷冻干燥、喷雾干燥、流化床干燥等^[8-9]。流化床干燥技术虽成本较低,但技术操作难度大,处理时间长,易使益生菌失活^[8]。喷雾干燥虽具有成本低和制备菌粉颗粒更为细腻的优点,但在维持益生菌活力方面逊色于真空冷冻干燥^[10]。真空冷冻干燥虽优势明显,但冻干过程会造成细胞的部分损

伤,从而影响益生菌应用中的活性。本文归纳梳理真空冷冻干燥对细胞损伤的机制,总结益生菌抗冻干损伤方法的最新研究进展,为开发高活性的菌粉产品提供理论参考。

1 冷冻干燥致益生菌损伤的原因解析

冷冻干燥对益生菌造成的损伤主要表现为细胞膜流动性降低与通透性变大,酶活性减弱,蛋白变性以及 DNA 双螺旋结构的破坏^[11-13](图 1)。这些损伤严重影响细胞的正常生长、代谢与繁殖能力,甚至可能导致细胞死亡。

1.1 对益生菌细胞膜的损伤

在冻干过程中,细胞膜的完整性受到损害,流动性降低,通透性变大。研究人员在对冻干后的微生物细胞膜进行检测时,观察到冻干后的微生物细胞膜 DPH(1,6-二苯基-1,3,5-己三烯)、荧光偏振(P)、各向异性(r)和微观黏度(η)值显著增加,表明细胞膜的流动性降低^[14]。冻干过程中的低温和脱水可能导致细胞膜中的脂质双层由液晶态转变为凝胶态(图 2),并且酰基将其头部基团上水分子替代,这些改变将导致细胞膜通透性和链间作用力变大^[15]。此外,细胞膜的再水合能力也可能因此受到损害,这将影响细胞在复水时能否恢复其功能。

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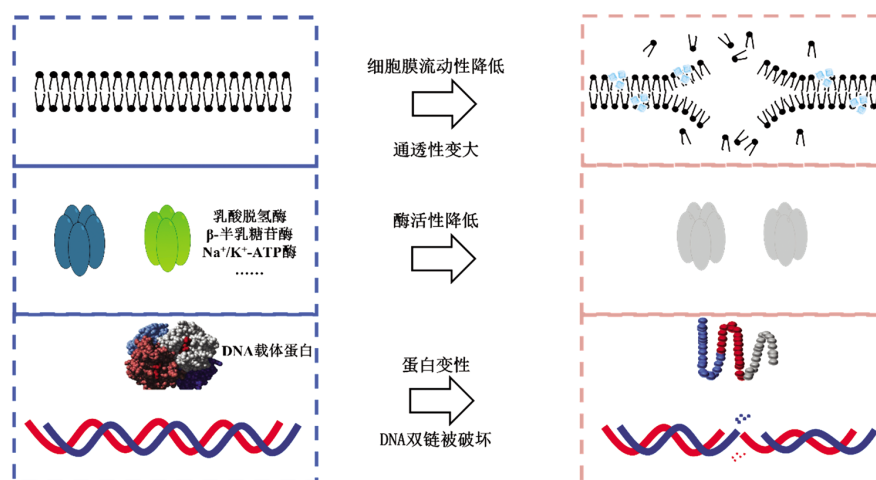


图 1 冷冻干燥对细胞造成的损伤

Fig.1 Cell damage caused by freeze-drying

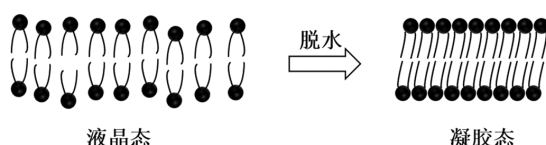


图 2 磷脂分子的状态转变

Fig.2 State transition of a phospholipid molecule

1.2 对益生菌酶的损伤

酶对温度和水分含量非常敏感，低温和脱水可能导致酶活性降低或完全失活，严重影响细胞的生长代谢与物质交换。乳酸脱氢酶(LDH)负责转化糖酵解终产物丙酮酸酶，其对冻干过程高度敏感，是评估冷冻干燥对细胞损伤程度的标志酶。冷冻干燥还对β-半乳糖苷酶（细胞衰老标志酶，负责控制乳糖消化酶）以及Na⁺/K⁺-ATP酶（负责维持细胞体积和渗透平衡，维持细胞膜电位，参与细胞信号传导以及调节跨膜离子梯度）产生不同程度的损伤，从而破坏蛋白构象，丧失其功能和活性^[16-18]。

1.3 对益生菌 DNA 的损伤

DNA 是遗传信息的载体，是生物多样性的基础，也是生物进化的关键。它对细胞的分类和生长起指导作用。益生菌中 DNA 常与载体蛋白以复合体形式存在。在冻干过程中，脱水可能会导致载体蛋白变性以及 DNA 损伤。有研究表明，在脱水过程中，DNA 双螺旋结构发生变化是导致瑞士乳杆菌死亡的主要原因^[13]。

脱水会增加溶质浓度，改变电荷分布，破坏 DNA 双螺旋结构中碱基对之间的糖苷键和氢键，从而引发脱嘌呤和脱嘧啶反应，最终影响核苷酸的组成^[19]。与此同时，载体蛋白在冷冻干燥过程中也会发生变性，这进一步导致 DNA 双螺旋结构的稳定性下降。从而严重影响了遗传物质的转录、翻译和复制，导致细胞活力下降^[20]。

2 基于调控培养基组分与培养条件的抗冻干损伤方法

不同益生菌对营养成分的种类和含量偏好有所差异。在益生菌培养过程中，调控培养基组分与培养条件，可以使细胞酶活力与发酵能力显著增强，细胞密度增加。并且有助于提高细胞膜的流动性，维持细胞壁完整性，刺激生物被膜合成上调（图 3），改变菌株形态，从而增强菌株对不良环境的抵抗能力。

2.1 培养基组分

2.1.1 碳源 研究报道，培养基中的碳源与细胞膜合成密切相关，过高或过低浓度的碳源均会抑制细胞膜形成^[21]。目前，葡萄糖是菌株培养过程中使用最多的碳源。然而，有研究表明，在培养基中适量添加蔗糖不仅能够提升菌株的抗冻干能力，还能促进菌株增殖，并增加培养期间胞外多糖的积累，从而增厚细胞壁^[22-23]。此外，蔗糖的凝胶特性使其在冷冻干燥过程中形成保护膜，从而维护细

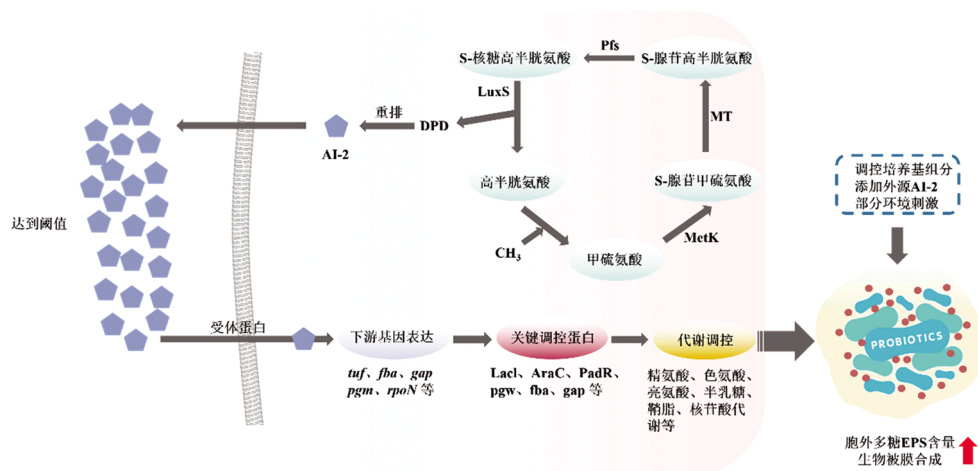
胞结构^[24]。许多学者研究认为海藻糖是一种高效的保护剂。在培养基中添加海藻糖,或者将其作为冻干保护剂,均可以显著抑制冰晶形成,其特有的高黏度和低流动性保护了细胞膜和蛋白质结构,从而显著提高菌株的冻干存活率^[14,25-26]。此外,还有研究报道,在培养保加利亚乳杆菌的过程中,使用复配碳源与单一碳源(葡萄糖)相比,菌株的冻干存活率显著增强^[27]。

2.1.2 氮源 氮源是影响菌体生长繁殖速度和代谢的重要营养物质,能够增强酶活性,并可能改变菌株的形态。其种类和含量显著影响益生菌的冻干存活率。例如:Shao等^[28]在高浓度的酵母抽提物(4%)培养基中培养保加利亚乳杆菌,结果显示,冷冻干燥死亡率高达88%,原因可能是较高浓度的酵母抽提物使菌株的比表面积扩大,冰晶更易刺穿细胞膜而导致细胞死亡。Gaucher等^[29]调节弗氏丙酸杆菌的生长条件,发现改变碳氮比可调节渗透保护剂的积累,刺激生物被膜的形成,提升该菌对不同压力的耐受性。Senz等^[30]观察到,在培养基中添加蛋白胨,并在过程中适当加热,能够将菌株的形态从长杆改变为短粗杆,并发现菌株形态与微生物的稳定性之间存在相关性。冻干过程中,短细胞可能比长细胞更为稳定。

2.1.3 氨基酸 氨基酸是益生菌生长和代谢的重要原料。菌株可以利用氨基酸执行多种生理功能,例如:调节细胞内pH值、能量代谢和渗透压等^[31]。E等^[32]的研究发现,培养基中添加L-谷氨酸能够

加快菌株生长,调节pH值,促进*dnaK*和*secG*等基因的表达,增加DnaK蛋白水平,促进对细胞膜的锚定作用,增强细胞膜的完整性。同时,*murL*、*murD*和*vanY*基因的表达也增加,促进肽聚糖(PGN)的合成,维护细胞壁的完整性。还有研究报道,在植物乳杆菌的培养中,添加天冬氨酸提高了菌株在冻干过程中的存活率,减少了冷冻干燥过程中的应变损伤,增加了细胞壁中肽聚糖的含量,调节了细胞膜的脂肪酸组成,并通过提高细胞内的pH值来减少DNA损伤^[33]。同样,在长双歧杆菌培养基中添加脯氨酸,有助于保持细胞膜的完整性,减少关键酶的损伤,从而增强菌株的抗冻干能力^[34]。这些研究表明,特定的氨基酸能够对某些菌株产生积极作用,从而提高益生菌在冷冻干燥过程中的存活率。

2.1.4 金属离子 金属离子如 K^+ 、 Mg^{2+} 和 Ca^{2+} 已被证明在冷冻干燥过程中对益生菌具有保护作用。研究表明,在培养植物乳杆菌LIP-1时,增加培养基中 K^+ 的浓度可以促进群体感应关键基因*luxS*的表达,进而增加了下游信号AI-2的合成,上调生物被膜合成基因*cysE*的表达,为菌株提供保护层,减少外界不利环境对菌株的损伤,提高菌株在冷冻干燥过程中的存活率^[35-36]。还有研究表明,在培养某些菌株时,添加 Ca^{2+} 可以有效提升菌株冻干存活率,促进裂解蛋白和表面蛋白的积累,强化细胞壁,增强酶活性,对细胞膜、DNA和蛋白质提供保护,从而提升菌株的生存能力^[22-23,37-38]。此



注: MetK;S-腺苷甲硫氨酸合成酶;MT;甲基转移酶;Pfs;S-腺苷高半胱氨酸核苷酸酶;DPD(4,5-二羟基-2,3-戊二酮)。

图3 AI-2/LuxS QS系统介导益生菌形成生物被膜的可能机制^[37,49-50]

Fig.3 AI-2/LuxS QS system mediates probiotic biofilm formation^[37,43-50]

外,催化磷酸二酯键形成的酶机制需要2个 Mg^{2+} , Mg^{2+} 是DNA聚合酶催化活性所必需的辅因子。推测在冻干过程中, Mg^{2+} 有助于稳定核糖体^[39],从而增加细胞在冻干过程中的稳定性。

2.1.5 其它成分 何棕柏等^[40]的研究表明在植物乳杆菌LIP-1培养基中,添加低质量浓度油酸(C18:1 ω 9c)能够促进棕榈酸向环丙烷脂肪酸的转化,同时也诱导了饱和脂肪酸向不饱和脂肪酸的转化,增强了细胞膜的流动性。将荧光染料(碘化丙啶和SYTO9)加入菌悬液中,在荧光显微镜下观察,发现绿色荧光菌体(绿色荧光表示活菌,红色荧光表示死菌)的数量明显多于对照组,试验组的细胞膜完整性较好。值得注意的是,当不饱和脂肪酸过量时会加剧氧化,因此通过改变不饱和脂肪酸含量提升细胞膜流动性时,应控制在适当的范围内^[41]。此外,Liu等^[42]在培养基中添加葡萄籽粉(GSF)刺激双歧杆菌生物膜形成,经处理后对比含GSF的培养基的活细胞数显著高于无GSF的培养基,生物被膜提高了细胞在恶劣环境中的存活率。

2.2 培养条件

不同益生菌的抗冻干最适pH值、温度和培养时间有所差异。例如,Wang等^[51]研究了3个发酵温度(30,37,42℃)和3个发酵pH值(4.5,5,6),发现嗜酸乳杆菌在pH 5或30℃的条件下,具有更好的冷冻耐受性和酸化活性。这与不饱和脂肪酸与饱和脂肪酸的高比例,低C18:0含量,以及高C16:0和环状C19:0的相对浓度有关。Liu等^[52]研究发现,罗伊氏乳杆菌I5007在pH 5.7和37℃的发酵条件下,细胞密度最高,然而其冻干存活率仅为12.95%。而在47℃和pH 6.7的发酵条件下,细胞膜中不饱和脂肪酸与饱和脂肪酸比例的增加,提升了细胞膜流动性,从而增加了冻干存活率。Hernández等^[53]研究分析发现,目标菌株的冷冻干燥存活率与pH值呈线性关系,在pH值为6.5时,存活率高达80%,而在pH值为4.5时,存活率最低仅为40%。Tovilla coutiño等^[54]在不同温度(42℃和37℃)、不同pH值(4.8和5.8)及不同生长阶段(指数中期、指数末期和稳定初期)培养的德氏乳杆菌保加利亚亚种CFL1时发现,细胞在42℃、pH 4.8条件下生长并在指数末期收获的

细胞抗冻性增加,这可能是由于在此条件下培养至对数末期的菌株活力达到最高,从而增强了对冻干环境的抵抗力。还有研究报道,培养罗伊氏乳杆菌DSM17938时,在非抑制氧气水平下,控制氧气供应,细胞膜中的不饱和脂肪酸含量升高,提升了细胞膜的流动性,从而使菌株的抗冻干性能增强^[55]。这些研究表明,调控培养条件可能会增加细胞膜的不饱和脂肪酸和环丙烷脂肪酸含量,从而提升细胞膜的流动性,增强细胞的活性,使其更易对抗冻干环境^[51,56-57]。

3 基于优化离心条件的抗冻干损伤方法

益生菌在培育至一定时间后,冷冻干燥之前会进行离心浓缩。浓缩的目的是减少细胞悬浮液的体积,并获得高细胞浓度,以降低运输和贮存成本^[58]。离心参数会影响菌株的得率、损伤程度与耐寒性。例如,Streit等^[59]在17 000 \times g、30 min的离心参数下,发现保加利亚乳杆菌CFL1的耐寒性增加。离心过程中,菌体受到的损伤与离心时间和离心力成正比。当离心时间短,离心力低时,菌体未完全沉淀,损失率较高;而当离心时间长,离心力较大时,会对菌体造成机械损伤,因此也有研究人员通过添加离心保护剂来降低损伤^[60-61]。探究目标菌株最优的离心条件有利于降低细胞损失率,减小机械损伤及增加耐寒性,从而提升菌株的冻干存活率。

4 基于胁迫预处理的抗冻干损伤方法

逆境胁迫是通过对菌株施加单一或多种环境压力,激发其适应性反应。这种方法不仅提高了菌株在恶劣环境中的生存能力,还通过交叉保护作用,即一种胁迫对另一种胁迫产生了保护效果,增强了菌株对多种逆境的耐受性,启动了菌株的自我保护机制(表1),提高了抗逆性^[62]。目前,研究中主要的胁迫方式包括酸胁迫、冷/热胁迫、渗透胁迫和其它胁迫。

4.1 酸胁迫预处理

酸胁迫可能通过以下几个方面提升菌株的冻干存活率:1)影响糖酵解途径;2)影响脂肪酸代谢途径;3)上调应激蛋白的表达^[63]。E等^[60]通过转录组学和蛋白组学发现,在酸胁迫条件下,菌株通过

上调乳酸脱氢酶基因的表达,促进丙酮酸向乳酸转化,从而增强能量供应,提高其适应恶劣环境的能力。为减轻长期酸性环境对生长的抑制作用,该菌株通过上调脂肪酸合成相关基因和蛋白质的表达,提高环丙烷和不饱和脂肪酸的相对含量,进而维持了细胞膜的完整性和流动性。此外,长期酸性环境激发了交叉应激耐受机制,显著上调了冷应激蛋白的表达^[56]。

4.2 冷胁迫预处理

冷胁迫处理后,菌体冷应激蛋白合成增加,细胞膜脂肪酸成分发生变化,提升了细胞膜的流动性,因此菌株对冻干环境的抵抗力增强^[64]。王晓萌等^[65]将嗜酸乳杆菌 ATCC4356 进行冷胁迫处理,其冻干存活率比正常对照组提高了 17.56%。还有研究人员以低温胁迫下的保加利亚乳杆菌为目标菌株,通过实时荧光定量聚合酶链式反应技术检测冷应激蛋白的表达基因 *cspA* mRNA,发现其拷贝量增加了 3.34 倍,说明冷应激蛋白含量上升^[66]。CSPs 在低温下具有高稳定性和高活性,能保护细胞膜上的相关酶,维持催化活性,增强细胞的抗冻干能力^[67-68]。

4.3 热胁迫预处理

热胁迫增强菌株抗冻干能力可能与加快恢复冷干干燥后的生长发酵能力,提高葡萄糖转化率与胞外多糖产量,以及促进热休克蛋白的表达有关。热休克蛋白能够促进蛋白质的正确折叠,帮助恢复变性蛋白质和新生多肽的结构与功能,从而保持菌株的代谢功能更加稳定^[69-71]。何亚婷等^[72]研究发现,以葡萄糖为保护剂结合热休克处理,对菌株正向叠加效果最显著,嗜酸乳杆菌的存活率从 34.42% 提高到 76.28%。Zhen 等^[73]对嗜酸乳杆菌进行 45 °C 热激处理 30 min,显著提高了菌株的 Na⁺/K⁺-ATP 酶活性以及胞内葡萄糖利用率,增加了乳酸和多糖的产量,存活率从 39.1% 提高到 56.3%。

4.4 渗透胁迫预处理

渗透胁迫通过过表达相容性溶质摄取系统诱导应激适应,导致其积累,从而增强冻干的耐受性,渗透压还可以通过添加盐和相容性溶质(如海藻糖、甜菜碱和脯氨酸等)来诱导^[9]。如高效冻干保护剂海藻糖,便可通过渗透胁迫积累在细胞内,而提升冻干存活率。还有研究表明唾液乳杆菌中过

表达 BteL(甜菜碱摄取系统)导致甜菜碱的积累,在冻干过程中提高抗性^[74]。Zhao 等^[75]发现,编码相容性溶质转运蛋白的基因在盐胁迫下也被上调。此外,Wang 等^[76]将菌株暴露于盐胁迫后,植物乳杆菌 LIP-1 细胞渗出过多的 Na⁺至细胞外,将细胞外 K⁺转运入细胞,导致与 K⁺转运相关的 *trkA* 基因上调,从而显著上调 *lysR* 型转录因子的表达,使细胞膜不饱和脂肪酸含量增加,降低细胞膜损伤程度,提高了冻干存活率。同时试验证明间接施用含有 NaCl 的磷酸盐缓冲液与直接施加盐胁迫相比,会导致更高的细胞内 pH 值和 ATP 含量,以此有效降低了 DNA 和细胞膜损伤。

4.5 其它胁迫预处理

Nguyen 等^[77-78]在试验中,将双歧杆菌暴露于环境胁迫中,进行了 CO₂ 刺激处理,结果显示该菌产生了更多的胞外多糖(EPS),并且证明 EPS 与冻干存活率之间呈正相关性。胞外多糖可与冻干保护剂形成双层微封装结构保护细胞,使细胞受到外部损伤降低。还有研究人员利用胆盐或营养胁迫促使细胞膜脂肪酸含量和比例发生变化,并且维持了胞内 β -半乳糖苷酶活性,进而提高了冻干存活率^[79-80]。

有效的胁迫预处理使菌株在冻干过程中的存活率提高,其作用机制可归结为:应激蛋白的产生,调控能量和膜脂肪酸代谢,积累相容性溶质等,来应对环境压力。其中应激蛋白的表达增加对抵御致命性应激至关重要,这种上调的蛋白表达有助于菌株在面对极端环境时保持活力和稳定性^[81]。然而,适应性胁迫仍会对细胞造成损伤,其在适应期的遗传物质难以预测,遗传稳定性无法保证^[82]。

5 基于冻干保护剂添加的抗冻干损伤方法

5.1 冻干保护剂

为减轻极低温和脱水等严苛条件对细胞的损害,预先添加保护剂是一种有效方法。研究显示,保护剂可以增加未冻结区域,并防止冰晶挤压造成的损伤^[90]。大多数单一的保护剂并没有较好的保护效果,因此在实际应用中通常会将多种保护剂复配以提高冻干存活率。寻找目标菌株适合的保护剂组合(表 2)是提高其冻干存活率的关键。

表 1 益生菌胁迫处理的适应性机制

Table 1 Adaptive mechanisms in probiotic stress treatment

益生菌	胁迫处理条件	适应机制	参考文献
嗜酸乳杆菌	45 °C, 30 min	Na ⁺ /K ⁺ -ATP 酶活性 ↑	[73]
干酪乳杆菌	45 °C, 4 h	PFK-6 ↑, PFK ↑, ENO ↑, FBA ↑, TPI ↑, GAP ↑, PGK ↑	[83]
克非诺氏乳杆菌	52 °C, 2 h	FOF1-ATPase ↑, DnaK ↑, GroEL ↑, ClpX ↑	[81]
短乳杆菌	-5 °C, 2 h	表层蛋白 ↑	[84]
德氏乳杆菌	10 °C, 2 h	cspA ↑, cspB ↑	[28]
植物乳杆菌	5 °C, 6 h	cspP ↑, cspL ↑	[85]
植物乳杆菌	pH 6.8	UFA ↑, LDH ↑, CspA ↑	[56]
鼠李糖乳杆菌	pH 4.8	F ₀ F ₁ -ATPase ↑	[86]
嗜酸乳杆菌	pH 5	UFA/SFA ↑	[51]
嗜酸乳杆菌	0.6 mol/L NaCl	表层蛋白 ↑	[87]
德氏乳杆菌	0.2 mol/L NaCl	甜菜碱 ↑	[88]
植物乳杆菌	碳水化合物饥饿	转为氨基酸代谢	[80]
植物乳杆菌	锰饥饿	UFA/SFA ↑, CFA ↑	[89]

注: ENO: 烯醇化酶; FBA: 果糖二磷酸醛缩酶; GAP: 甘油醛-3-磷酸脱氢酶; PFK: 磷酸果糖激酶; PFK-6: 6-磷酸果糖激酶; PGK: 磷酸甘油酸激酶; TPI: 磷酸丙糖异构酶; CFA: 环脂肪酸; UFA: 不饱和脂肪酸; SFA: 饱和脂肪酸; LDH: 乳酸脱氢酶; CspA: 冷应激蛋白。

表 2 冻干保护剂对细胞的保护作用机制

Table 2 Protective mechanism of lyophilized protective agents on cells

冻干保护剂种类	保护作用机制	常见物质	参考文献
糖类	提高玻璃化转变温度; 与菌体蛋白质极性基团(或水分子和细胞膜中的磷酸基团)形成氢键; 抑制冰晶生成	蔗糖、海藻糖、半乳糖、功能性低聚糖	[22], [61], [91]~[96]
蛋白质类	提高玻璃化转变温度; 促进益生菌细胞蛋白的氨基和羧基与保护剂之间的相互作用; 形成对细胞的保护层, 降低渗透压差, 并且在细胞表面形成多孔结构, 促进细胞复水; 调节渗透压	脱脂乳、乳清蛋白、胶原蛋白、大豆分离蛋白、大米蛋白、重组抗冻蛋白	[22], [69], [91], [92], [97]~[101]
肽类	提高玻璃化转变温度; 形成玻璃状基质(较高黏度), 固定益生菌细胞; 天然肽类聚合物β-折叠结构中的氢键提供的疏水相互作用; 抑制冰晶形成	肌肽、谷胱甘肽、胶原蛋白肽、乳清蛋白水解物、丝素蛋白、抗冻多肽	[102]~[111]
氨基酸类	提高玻璃化转变温度; 与胞内蛋白质氨基结合, 稳定蛋白结构; 抑制冰晶生成; 调节丙酮酸含量	谷氨酸钠、半胱氨酸	[23], [112]
醇类	醇类中的羟基可以代替蛋白质表面水分子的羟基, 从而在蛋白质表面形成一层水化膜; 抑制冰晶生成	甘露醇、甘油	[113], [114]
无机盐类	调节渗透压	乙酸钠、硫代硫酸铵	[115], [116]
脂肪酸类	提升了不饱和脂肪酸与饱和脂肪酸比率	油酸	[17]
生物类	提升胞外多糖含量	微藻生物	[117]

表 3 冻干保护剂在益生菌中的应用

Table 3 Application of lyophilization protectants in probiotics

益生菌	冻干保护剂	冻干存活率/%	参考文献
鼠李糖乳杆菌	11.1%海藻糖、9.1%甘油、3.5%谷氨酸钠、15.7%脱脂乳	97.8	[16]
瑞士乳杆菌 MB2-1	300 g/L 低聚半乳糖	77.89	[61]

(续表 3)

益生菌	冻干保护剂	冻干存活率/%	参考文献
植物乳杆菌 TISTR 2075	10%大米蛋白和 5%低聚果糖	71.34	[91]
动物双歧杆菌 MG741	1%谷胱甘肽	86.52	[110]
乳酸乳球菌 ZFM559	4.2%海藻糖、2.0%甘露醇、11.9%脱脂乳、4.1%谷氨酸钠	81.02 ± 0.32	[114]
双歧杆菌 BB01	甘氨酸 5.5%、碳酸氢钠 0.8%、低聚木糖 7%、精氨酸 4.5%、脱脂乳 25%	90.37 ± 1.9	[118]
植物乳杆菌 L1	10%脱脂乳、13%蔗糖、2%山梨醇、0.8%酪氨酸	97.4	[119]

基于目前益生菌冻干保护剂研究工作,对于不同益生菌冻干保护剂筛选研究工作的耗时较长、保护剂成本较高、保护效果不理想等问题,未来可开发普适性较强的新型冻干保护剂。若其还可与益生菌形成合生元,则会使益生菌产品的效果进一步提升。如 Wu 等^[120]系统研究了由低聚半乳糖(GOS)和罗伊氏乳杆菌组成的合生元,在小鼠结肠炎模型中的治疗潜力,揭示了 GOS 和罗伊氏乳杆菌通过促进五癸酸(一种奇链脂肪酸)的合成,协同保护肠道炎症和屏障功能障碍。

5.2 菌泥与保护剂配比

冻干保护剂与菌泥的比例对益生菌的冻干存活率有显著影响。如果保护剂的浓度过低,将无法为菌株在真空冷冻干燥过程中提供足够的保护,从而影响其活性;如果保护剂浓度过高,则可能导致细胞冻干存活率降低。这可能是由于高浓度的保护剂会妨碍水分的蒸发,进而促进冰晶的形成,增加细胞的死亡风险^[121]。

6 基于冷冻干燥工艺优化的抗冻干损伤方法

冷冻干燥主要分为 3 个阶段:预冻阶段、升华干燥(1 次干燥)和解析干燥(2 次干燥)。真空冷冻干燥机通过运行真空泵,使内部空间保持在低压状态,并向样品提供能量,从而使水分从固态直接跃升为气态^[122]。

6.1 预冻阶段

益生菌被冷却至冰点以下的过程中,会产生冰晶。冰晶的大小与冷却速率成反比。较大的冰晶在干燥产品中留下较大的孔径,因此它们对质量传递的阻力较低。相比之下,较小的冰晶留下较小

的孔径,这显著阻碍了水蒸气的传输。因此,干燥产品对质量传递的阻力与冻结步骤中形成的冰晶大小成反比^[12],这说明冰晶过小可能会增加后续干燥阶段仪器运行时间。

冰晶的大小决定着对菌株的损伤程度。当冷却速度过慢时,细胞可能因水分过度流失而发生收缩;相反,如果冷却速度过快,细胞内外的水分子可能会迅速结冰,形成冰晶。这些冰晶可能会穿透细胞结构,对细胞造成严重的物理性损伤^[123]。Wang 等^[124]研究发现植物乳杆菌 ST-3 与干酪乳杆菌 LC2W 的最佳冷却速率分别为 $-1\text{ }^{\circ}\text{C}/\text{min}$ 和 $-10\text{ }^{\circ}\text{C}/\text{min}$ 。Yang 等^[125]研究表明,迅速冷冻至 $-80\text{ }^{\circ}\text{C}$ 比缓慢冷冻至 $-20\text{ }^{\circ}\text{C}$ 的冻干存活率显著提高。Ming 等^[126]研究发现, $-80\text{ }^{\circ}\text{C}$ 预冻的唾液乳杆菌 I24 的冻干存活率高于 $-30\text{ }^{\circ}\text{C}$ 预冻的存活率。显然,冻结速率与冻结温度会影响细胞的冻干存活率,因此根据菌株的特点选择合适的冷却速率和预冻温度,可以减少细胞所受到的损伤并降低运行成本。此外,冰晶的大小对样品的状态(孔隙率和表面)与复水能力也有显著影响^[22]。

6.2 升华干燥(1 次干燥)

升华干燥(第 1 次干燥),样品中大部分水被去除,冰被转化为水蒸气,样品表面首先被干燥,逐步形成增厚的样品层^[127]。若设置较低的升华温度,相应的会有较长的干燥时间,可能导致结合水的去除和蛋白质结构的破坏^[128]。提高升华温度,可减少干燥时间,有利于菌种的存活,而温度应低于玻璃化转变温度,以防止应变坍塌^[22]。Sang 等^[129]研究表明,当长双歧杆菌 BB68S 的升华温度为 $-10\text{ }^{\circ}\text{C}$ 时,其胞内 β -半乳糖苷酶、LDH、 Na^+/K^+ -ATP 酶和 $\text{Ca}^{2+}/\text{Mg}^{2+}$ -ATP 酶活性显著高于 $0\text{ }^{\circ}\text{C}$ 和 $-20\text{ }^{\circ}\text{C}$ 的

结果。另外低气压也可以加快升华速度,而过低的气压反而不利于热量传导。因此达到减少益生菌损伤、缩短冻干时间和降低运行成本的目标需要综合考虑^[130],控制温度和压力在适当范围内调节。与预冻阶段相比,此过程的优势在于能够通过调节传热和传质条件,更容易地控制样品状态^[131]。

6.3 解析干燥(2次干燥)

升华干燥结束后,自由水已全部去除,残余水分吸附在样品表面,并可能以水合物结晶的形式存在,或形成无定形基质或玻璃态。此阶段,固体表面的水蒸气压力降低,干燥速度明显下降,可能需要适当提高搁板温度,以促进水分蒸发。通常对于菌粉产品而言,残余水分小于5%,水分活度在0.1~0.25之间,可以达到贮藏要求^[132]。低水分活度虽可减缓细胞代谢,延长保质期,但较低水分活度可能无法满足细胞结构的维持,若当水分活度小于0.1时,膜上的磷脂更易被氧化,这显然不利于产品贮藏^[133]。

6.4 物料厚度

物料厚度是影响残余水量和冻干存活率的重要因素。厚度与升华干燥结束时的残余水量成反比。适当增加物料厚度会延长解析干燥时间,从而导致较低的残余水分^[134]。而过长的干燥时间并不利于菌株存活。因此根据菌株的特点选择合适的冻干厚度,有利于提升益生菌的冻干存活率^[121,135]和获得较长保质期的益生菌菌粉。

7 结论与讨论

随着食品和医药行业的蓬勃发展,消费者对益生菌产品的需求日益增加,利用真空冷冻干燥技术来生产高活性的益生菌产品是行业的重要目标。本文对现有的研究进行综述,得出冷冻干燥主要对益生菌的细胞膜、酶活性、DNA等造成损伤,损伤程度取决于冰晶大小与形成速率。本文概述多种方法:1)优化冷冻干燥工艺控制冰晶大小和形成速率,来降低冰晶对细胞的损伤;2)调控培养基组分与培养条件刺激生物被膜合成上调,调控细胞膜不饱和脂肪酸;3)胁迫预处理诱导菌株产生自我保护反应;4)优化离心条件,提高菌体得率,降低机械损伤;5)添加冻干保护剂对益生菌封装保护,提高玻璃化转变温度。

为更好的满足消费者对产品需求,促进益生菌行业发展。基于目前研究现状,未来工作可聚焦于以下方面:鉴于目前不同益生菌对冻干保护剂的需求并不一致,保护剂筛选工作繁杂,保护效果并不理想,未来需要加强对新型保护剂(普适性较强)的开发。胁迫预处理虽可加强益生菌对极端环境的耐受力,但适应期对益生菌的损伤及其对遗传物质稳定性的影响也应进一步阐明。目前的研究多集中于利用单一的方法来提升冻干存活率,未来应深入研究多种方法相结合,以及它们之间的相互协作机制,进一步提高冻干存活率。冻干之后益生菌发酵活性、益生性能以及遗传物质是否发生变化也应进一步解析,以及如何将研究成果应用到工业生产的实际环境中更需详细考虑。

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Research Progress on Probiotic Anti-Freeze-Drying Injury Methods

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Abstract In recent years, probiotic products have been widely applied in the food, medical, and healthcare industries. However, maintaining strain viability during industrial production, transportation, and storage remains a challenge. Currently, enterprises and research institutions primarily employ dehydration to maintain probiotic activity. Among these methods, vacuum freeze-drying has become the mainstream choice as it can maintain strain viability to the greatest extent with low energy consumption and a simple process. Nevertheless, vacuum freeze-drying still causes damage to the cell membranes, enzymes, and DNA of probiotics, reducing their activity during application. This paper summarizes the latest research progress in methods to resist freeze-drying damage in probiotics, namely by regulating media components and cultivation conditions, stress pre-treatment, optimizing centrifugation conditions, adding cryoprotectants, and optimizing freeze-drying processes to enhance the freeze-drying resistance of strains and reduce freeze-drying damage. This work aims to provide a theoretical reference for the preparation of high-viability probiotic products.

Keywords probiotics; freeze-drying; damage; methods; activity