2025, 65(2): 421-436
CSTR: 32112.14.j.AMS.20240596
DOI: 10.13343/j.cnki.wsxb.20240596

微生物学报
Acta Microbiologica Sinica
http://journals.im.ac.cn/actamicrocn

Review 综述

乳酸菌在不同胁迫下应激反应及高活性保护机制研究进展

陈宁[1,2]，吴磊[2]，谢新强[2]，赵昕宇[3*]，吴清平[2*]

1 华南农业大学 食品学院，广东 广州
2 广东省科学院微生物研究所，华南应用微生物国家重点实验室，广东省微生物安全与健康重点实验室，国家卫健委微生物食品营养与安全科技创新平台，广东 广州
3 广东科环生物科技有限公司，广东 广州

陈宁, 吴磊, 谢新强, 赵昕宇, 吴清平. 乳酸菌在不同胁迫下应激反应及高活性保护机制研究进展[J]. 微生物学报, 2025, 65(2): 421-436.
CHEN Ning, WU Lei, XIE Xinqiang, ZHAO Xinyu, WU Qingping. Research progress in responses and high activity protection mechanisms of lactic acid bacteria under different stresses[J]. Acta Microbiologica Sinica, 2025, 65(2): 421-436.

摘　要：乳酸菌在食品和医学领域应用广阔，在食品发酵行业中可用作发酵剂和功能性菌粉。乳酸菌的活性和作用效果会受到各种环境压力的影响，如渗透压、温度、氧、酸、胆盐等。可食用的乳酸菌能够发挥多种多样的健康疗效，但其活菌数在生产、储藏以及人体消化过程中均有减少。因此，本文系统阐述了乳酸菌在其生产、储藏和消化过程中遭受的不同胁迫环境以及面对环境压力的应激反应，并从隔离胁迫环境和增加菌株抗逆性 2 个方面总结了现有高活性保护策略与机制，以期为乳酸菌菌株的选育与产品开发应用提供必要的理论依据。

关键词：乳酸菌；胁迫；应激反应；保护机制

资助项目：国家重点研发计划(2023YFF1000302)；广东省自然科学基金(2024A1515010621)；广东省科学院项目(2022GDASZH-2022010101)
This work was supported by the National Key Research and Development Program of China (2023YFF1000302), the Natural Science Foundation of Guangdong Province (2024A1515010621), and the Project of Guangdong Academy of Sciences (2022GDASZH-2022010101).
*Corresponding authors. E-mail: WU Qingping, wuqp203@163.com; ZHAO Xinyu, zhaoxy9897@163.com
Received: 2024-09-27; Accepted: 2024-11-10; Published online: 2024-12-23

Research progress in responses and high activity protection mechanisms of lactic acid bacteria under different stresses

CHEN Ning[1,2], WU Lei[2], XIE Xinqiang[2], ZHAO Xinyu[3*], WU Qingping[2*]

1 College of Food Science, South China Agricultural University, Guangzhou, Guangdong, China

2 State Key Laboratory of Applied Microbiology Southern China, Guangdong Provincial Key Laboratory of Microbial Safety and Health, National Health Commission Science and Technology Innovation Platform for Nutrition and Safety of Microbial Food, Institute of Microbiology, Guangdong Academy of Sciences, Guangzhou, Guangdong, China

3 Guangdong Kehuan Biotechnology Co., Ltd., Guangzhou, Guangdong, China

Abstract: Lactic acid bacteria (LAB) have extensive applications in food and medicine fields. They are used as starters and functional probiotics in food fermentation. The activity and performance of LAB are influenced by various environmental stresses involving osmotic pressure, temperature, oxygen, acidity, and bile salts. Edible LAB can provide numerous health benefits. However, their viable counts decrease during production, storage, and digestion. This paper systematically discusses the different stressful environments faced by LAB during production, storage, and digestion, as well as their stress responses. Furthermore, this paper summarizes existing high activity protection strategies and mechanisms from two aspects: isolating from stressful environments and enhancing strain resistance. This review aims to provide theoretical support for LAB strain engineering and product development.

Keywords: lactic acid bacteria; stress; stress response; protection mechanism

乳酸菌(lactic acid bacteria, LAB)是一类无芽孢的革兰氏阳性菌株，它们通过发酵产生大量活性物质，这些物质有助于促进健康，被广泛应用于发酵食品中[1]。在食品工业中广泛使用的LAB主要有链球菌属(*Streptococcus*)、双歧杆菌属(*Bifidobacterium*)、乳杆菌属(*Lactobacillus*)、乳球菌属(*Lactococcus*)、酒球菌属(*Oenococcus*)和魏斯氏菌属(*Weissella*)等[2]。LAB可以调节肠道菌群[3]、降低胆固醇、改善睡眠以及提高人体免疫力[4]。本团队已挖掘出多株有自主知识产权且具有良好益生功能的LAB[5-7]，其中Wu等[8]从百岁老人肠道微生物中筛选出一株可以产抗坏血酸的具有抗氧化功能的植物乳杆菌，Yang等[9]通过动物实验表明，副干酪乳杆菌可以缓解大鼠的高胆固醇血症，还有一株高产γ-氨基丁酸的植物乳杆菌能够提高睡眠障碍人群的睡眠质量[10]。LAB的生物转化产品在食品工业中得到了广泛应用，以功能性食品的形式食用可以改善人体健康并有助于预防疾病[11]。然而，LAB在食品发酵和生产过程中遇到了各种非生物和生物胁迫，严重影响其工业化生产效率和功能活性的发挥[2]。LAB在不同环节与用途所遭受的胁迫也不尽相同。在用作发酵剂时，LAB必须在生产和储存过程中抵抗离心、干燥等工艺程序带来的胁迫，而当用作活疫苗或益生菌菌粉时，LAB则需要承受胃肠道的压力[12]。隔离外界胁迫是工业上保护LAB活性最高效与经济的方法之一。Chen等[13]发现重组抗冻肽可

以抑制嗜热链球菌细胞凋亡，提高其冷冻胁迫下存活率。Liu 等[14]设计出具有 pH 响应和黏接性能的硫代氧化魔芋葡甘露聚糖微球，可以使 LAB 免受胃酸的伤害。除此之外，还有许多研究证明，LAB 为了应对不同类型的压力自身也开发了多种保护策略来应对外界的恶劣条件和环境的快速变化[15-17]。通过合成分子伴侣、酶、抗氧化剂和胞外多糖等特殊的生物分子，可以增强 LAB 对外界环境胁迫的耐受性[18]，并且能够在显著提高自身于应激条件下的耐受性的同时不会损害其生理活性[19]。因此，如何提高 LAB 的抗胁迫能力成为解决生产问题的关键。

为扩大益生菌市场，满足消费者的需求，迫切需要开发和制备具有益生功能的 LAB 产品。环境胁迫不仅对于 LAB 工业应用至关重要，而且会影响到菌株的生理活性及其益生功能。因此，本综述旨在总结不同环境胁迫下 LAB 的应激反应及其分子调控机制，从隔离胁迫环境和增加菌株抗逆性 2 个方面总结了现有高活性保护策略与机制，对 LAB 产品菌种的筛选和改良，以及生产工艺优化都具有重要指导意义。

1 LAB 受到的胁迫环境

LAB 产品从制备到消费，需历经多重环境考验，图 1 总结了 LAB 在生产、储藏、消化 3 个环节所面临的胁迫因素。

1.1 乳酸菌生产和储藏中受到的胁迫

1.1.1 乳酸胁迫

LAB 在高密度培养时会产生大量乳酸，很大程度上抑制了 LAB 的生长活力，是工业生产上无法避免的压力环境，其含量会随着 LAB 生长阶段的变化而变化[20]。乳酸会通过被动扩散进入细胞膜并解离成质子和酸残基，一旦它们进入细胞，则无法通过细胞膜返回外部，导致质子在细胞内积累，从而影响跨膜电位，进而

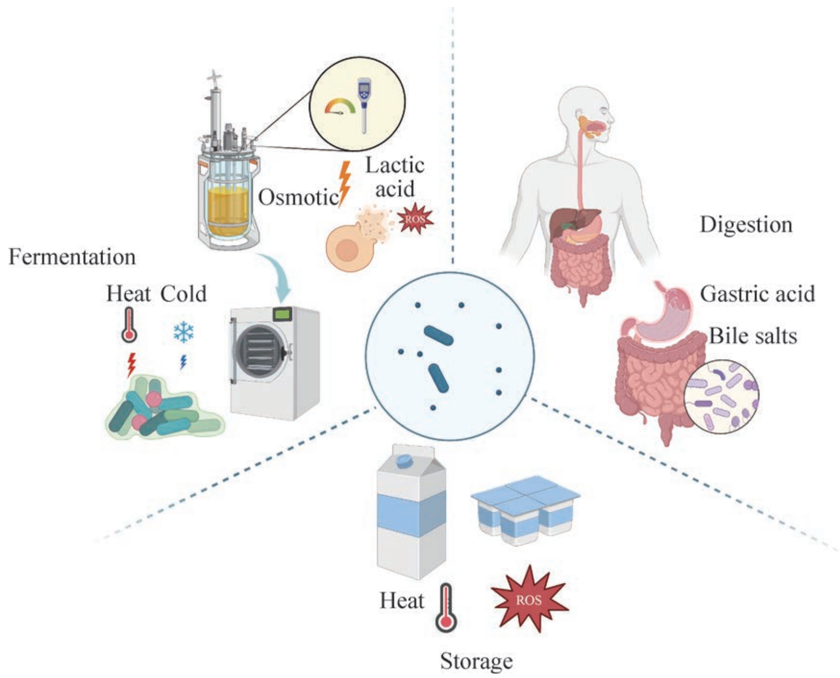

图1 LAB在不同环境下所受到的胁迫

Figure 1 Stress of LAB in different environments. Created with BioRender.com.

影响多种跨膜转运机制的能量来源[2]。Zhu 等[21]发现乳酸乳球菌中参与嘧啶生物合成途径和甘氨酸或甜菜碱转运过程的基因也会在酸胁迫期间上调。若在 LAB 对数生长阶段用较温和的酸性条件刺激，它们能够展现出启动适应性反应的能力，这一过程被称为酸耐受应激(acid tolerance response, ATR)[12]。例如，将 LAB 短暂地暴露于 pH 5.0 的环境中 1 h，再转移至 pH 2.0 的环境中，其活菌数量并未降低[22]。除上述适应性反应外，LAB 还能够利用 ATP 酶(也称 H^+-ATP 酶)来维持细胞内 pH 稳态，它通过水解 ATP 将 H^+泵出细胞，或利用 ATP 水解产生的能量从细胞中去除 H^+，在维持 pH 稳态中发挥重要作用[23]。除此之外，LAB 还可以通过氨基酸的脱羧反应，从细胞中吸收 H^+，然后通过细胞膜排出，使细胞内 pH 值升高[12]。

LAB 菌株生长过程中的乳酸环境较为常见，了解酸应激反应的分子机制有助于调整和改造这些 LAB 菌株用于工业应用。

1.1.2 渗透压胁迫

针对 1.1.1 所述的乳酸胁迫，工业上一般会采取流加碱液的方式来中和乳酸对菌体生长的反馈抑制，然而这种方法伴随大量盐分的产生，进而导致渗透压升高[20]。渗透压的提升是影响许多发酵食品生产的一个重要问题。首先，LAB 细胞的膜蛋白可直接或间接调节细胞膜对盐离子的渗透性，Na^+/H^+逆向转运蛋白会外排 Na^+，这不仅能减轻 Na^+对菌体细胞的毒害作用，而且能通过增加对 K^+的吸收来恢复 Na^+/K^+比[24]。为了更好地应对渗透压的变化，LAB 还会积累更多的相容性物质[25]，以此来平衡细胞内外渗透压，提高细胞的耐受阈值[12]，并减少细胞失水的风险。相容性溶质是 Brown 等[26]在 1972 年首次提出的可以在细胞中以高浓度聚集，而不妨碍细胞功能的蛋白质分子正确折叠的小分子或结构化合物[27]。目前报道的参与渗透胁迫应激的相容性物质有甘氨酸甜菜碱和胆碱等[28]。然而，不同种类的 LAB 在相容性分子的积累机制上也存在差异[29-30]。在植物乳杆菌中，渗透剂的吸收是通过对甜菜碱具有高亲和力并能够转运季铵盐化合物的 QacT 系统协调的[31]。相比之下，在乳酸乳球菌中，甜菜碱和脯氨酸的摄取是由 *opuA*/*busA* 操纵子控制，该操纵子编码 ABC 家族转运蛋白，通过消耗 2 个 ATP 分子来转运单个甜菜碱分子，转运蛋白的活性取决于细胞内离子强度的变化以及转运蛋白本身与细胞膜之间的静电作用[32]。

综上所述，LAB 在面对外界渗透压的改变时，一是依靠细胞膜对膜电位的维持，二是通过积累相容性溶质来适应。

1.1.3 温度胁迫

LAB 产品的细胞数量和活力是影响产品质量的关键因素[33]。冷冻干燥技术目前被认为是保持菌株产品活性的最佳干燥方式之一[34]。与其他干燥方式相比，冷冻干燥有很多优点，但同样地，由于细胞暴露于极冷环境，它也可能对细胞壁、细胞膜和 DNA 造成生理损伤，从而影响细胞的活力和功能活性[35]。LAB 细胞会诱导产生冷诱导蛋白减轻冷刺激造成的细胞损伤，该蛋白能调控低温胁迫下的细胞来保证正常 DNA 的超螺旋结构和增加膜质中短链或不饱和脂肪酸的比例，以维持细胞膜的流动性。尽管冷冻干燥可以长期保持 LAB 细胞的活力，但由于其成本高昂，在乳品行业中，喷雾干燥仍然是普遍的干燥方式，LAB 最适的生长温度在 30−37 °C之间[36]，而喷雾干燥过程中的高温会引起热应激，破坏细胞稳态，导致细胞中蛋白质的变性和聚集，以及影响 DNA 等生物大分子的稳定性和膜的流动性[37]。公丕民[38]的研究发现，喷雾干燥过程中细胞膜通透性的改变是导致细

胞死亡的主要原因。不仅细胞膜脂肪酸的组成在干燥之后会发生相变，细胞壁的修复功能也会受到抑制。对于商业益生菌的生产而言，随后的储存环境中的温度也是能否保证其活菌数量的关键条件，LAB 的短期保存以 4 ℃储藏为主，而长期储藏则以−20 ℃效果最佳[35]。Shu 等[39]的研究发现，由于常温下的菌粉胞内水分活度较高，而低温储藏细胞生长代谢较慢，因此常温储藏会更快丧失活力。此外，研究表明，当储藏温度高于玻璃化转变温度时，LAB 产品会呈现橡胶态，导致接触时间减少，从而更容易发生结块现象[40]。

因此，研究冷应激以及热应激可以充分了解 LAB 对不同应激条件的适应性，是之后开发相应保护策略来保证在食品生产和储藏环境中质量和功能的重要一步。

1.1.4 氧胁迫

LAB 最终产品的总活菌数量与活力对产品的质量与益生功效至关重要，储藏环境的高氧气含量不仅会降低菌株的酶活性，还会促进不饱和脂肪酸的氧化，此外，LAB 在生长代谢过程中产生的活性氧(reactive oxygen species, ROS)也会对菌体的蛋白质和 DNA 造成较大损伤，从而降低细胞活性[41]。活性氧广泛指氧来源的自由基和非自由基，由于它们含有不成对的电子，因此具有很高的化学反应活性，例如超氧阴离子(O^{2-})、臭氧(O_3)和单线态氧($1O_2$)等，自然条件下，ROS 来源于胞内酶的自氧化、环境中的氧化还原反应、竞争性微生物的释放等[42]。LAB 与氧胁迫应激反应相关的酶主要有 NADH-氧化酶/NADH-过氧化物酶和超氧化物歧化酶(superoxide dismutase, SOD)，NADH-氧化酶/NADH-过氧化物酶系统可在 NADH 氧化酶作用下生成 H_2O_2，再通过 NADH 过氧化物酶将其分解为水，防止 LAB 细胞中氧气积聚[2]。Gibson 等[43]通过插入失活编码 NADH-氧化酶的基因来构建 NADH-氧化酶缺陷突变体，证明了其在碳水化合物和氧含量高的环境中至关重要。SOD 是 LAB 中最重要的抗氧化酶之一，它能使 O^{2-} 突变，从而降低细胞内游离金属阳离子的浓度，并有效减轻 H_2O_2 对细胞造成的损伤[44]。除了从抗氧化角度抵抗氧胁迫之外，维持细胞内的还原性也同样重要。研究表明，在添加谷胱甘肽的培养基中生长的 LAB 细胞，对 H_2O_2 处理的抗性显著增加，这可能是因为添加谷胱甘肽之后激活了细胞中的谷胱甘肽-谷胱甘肽过氧化物酶-谷胱甘肽还原酶系统[45]。Jänsch 等[46]也利用基因敲除的方法证明了 *gshR* 基因敲除株与野生型菌株相比，前者在改良的 MRS 培养基上的有氧生长速率显著降低。

综上所述，LAB 所受到的氧胁迫不仅来源于外界分子氧的攻击，其本身的菌株生长代谢过程中产生的活性氧也是一个不可忽视的重要来源。在储藏过程中，LAB 产品活性的降低不仅归因于氧的胁迫，储藏环境温度的改变也是一个至关重要的影响因素。因此，许多研究也着重于探究菌株的交叉保护机制，即探讨如何通过轻微胁迫下的预适应来提高 LAB 对其他环境胁迫的耐受性[2]。

1.2 消化过程中的胁迫

1.2.1 胃酸胁迫

消化道是抵抗外来病原微生物对机体损害的天然屏障，然而具有益生作用的 LAB 产品同样需要通过这一屏障来发挥作用。LAB 所面临的酸胁迫环境主要有两方面，一是在增殖过程中所产生的乳酸会影响菌体自身的生长和代谢，二是 pH 值接近 2.0 的胃酸[12]。胃酸是 LAB 进入人体后发挥益生作用所遇到的第一个胁迫环境，LAB 的益生作用的发挥取决于通过胃液之后所到达作用部位的活菌数量[47]。与菌株生长

代谢产生的乳酸不同，胃液的独特成分和低 pH 值对于 LAB 的生存来说是一个巨大的挑战，这归因于胃的酸性条件会在细胞水平上引起严重的形态和表型改变，例如微生物细胞膜组成的变化、DNA 的损伤以及肽聚糖成分的改变[48]。细胞膜是大多数细胞溶质交换的主要屏障，同时也是应激损伤的主要部位，对于维持细胞内外 pH 稳态至关重要。为了防止质子流入细胞，细胞通常会通过调节脂肪酸的组成来改变膜的流动性，例如在双歧杆菌中，细胞膜上较高比例的环丙烷脂肪酸与较长的脂肪酸链有助于降低膜的流动性，从而增强菌株的耐酸能力[49]。Wei 等[50]也发现，在低 pH 条件下，长双歧杆菌会通过重塑肽聚糖生物合成和脂肪酸代谢来修饰其细胞壁。另外，当 LAB 的 DNA 暴露于强酸中时，由于碱基的质子化以及糖苷键的断裂，会产生无嘌呤和无嘧啶位点[12]。

LAB 在遭受胃酸胁迫时，主要是通过加强细胞屏障防止外来质子流入造成细胞内外 pH 失衡。

1.2.2 胆盐胁迫

胆盐是胆汁中参与消化吸收脂肪的主要成分[51]，它通过溶解细胞膜中的磷脂和膜蛋白来达到杀菌的目的[52]。LAB 需要经过多重消化道之后才能到达肠道发挥益生作用，而消化道的上一级环境引起的 LAB 代谢变化可能会影响下一级环境中的生理功能[53]。为了维持自身稳态，LAB 细胞会通过调控膜蛋白的基因表达来控制外来胆盐的渗透率，在嗜热链球菌中被证明通过调控基因来保持细胞膜完整性从而增加对胆盐的抗性[54]。与此同时，LAB 为了克服胆盐胁迫，通过调整其能量代谢的途径、水平来维持稳态[2]。Sanchez 等[55]对长双歧杆菌的蛋白质组学与转录组学进行分析后发现，在胆盐胁迫下，参与转录和翻译以及糖酵解和丙酮酸分解代谢的酶表达量增加。乌日娜[56]也发现，干酪乳杆菌在含有 1.5% 胆盐的培养基中生长后，其应激蛋白、糖代谢蛋白等 26 种蛋白质的表达发生了变化。当受到胆盐刺激时，LAB 会产生能够将结合型胆盐转化成游离型胆盐的胆盐水解酶 (bile salt hydrolase, BSH)，来减少胆盐对细胞的毒害[51]。有研究将不含 BSH 与含 BSH 的 LAB 比较发现，含有 BSH 的 LAB 对共轭胆汁酸有更强的抵抗力[57]。Grill 等[58]也发现，无共轭胆汁盐水解酶活性的突变株的生长速率受胆盐的影响更大。上述结论表明，LAB 在面对胆盐胁迫时具有多种适应性机制和策略。

2 乳酸菌在不同胁迫环境下的应激保护策略及保护机制

不同环境对 LAB 的胁迫有所不同，从对恶劣环境进行隔离和增强菌株自身抗逆性 2 个方面来设计不同的保护策略能够具有针对性和可靠性。图 2 总结了目前 2 个方面的应激保护策略。

2.1 隔离胁迫环境

2.1.1 添加保护剂

LAB 通常以菌粉的形式用作膳食补充剂[59]、奶酪或酸奶生产的发酵剂。然而，LAB 发酵剂很难避免冷冻和干燥过程中的各种损害。因此，可以选择添加合适的保护剂来提高 LAB 在生产时的存活率和活力[60]。吴家琳等总结了常用的冻干保护剂有糖类、蛋白质类、盐类、高分子类等[61]，其中糖类保护剂研究最为广泛，特别是非还原性二糖，例如海藻糖和蔗糖，这归因于它们较高的玻璃化转变温度更适于低温保存[62]。Ge 等[63]的研究也佐证了由于玻璃化转变温度的提高，有效地减轻了在冻干过程中由冰晶引起的机械损伤，并保持细胞膜上 Na^+/K^+-ATP 酶的活性。海藻糖、蔗糖和脱脂乳的组合

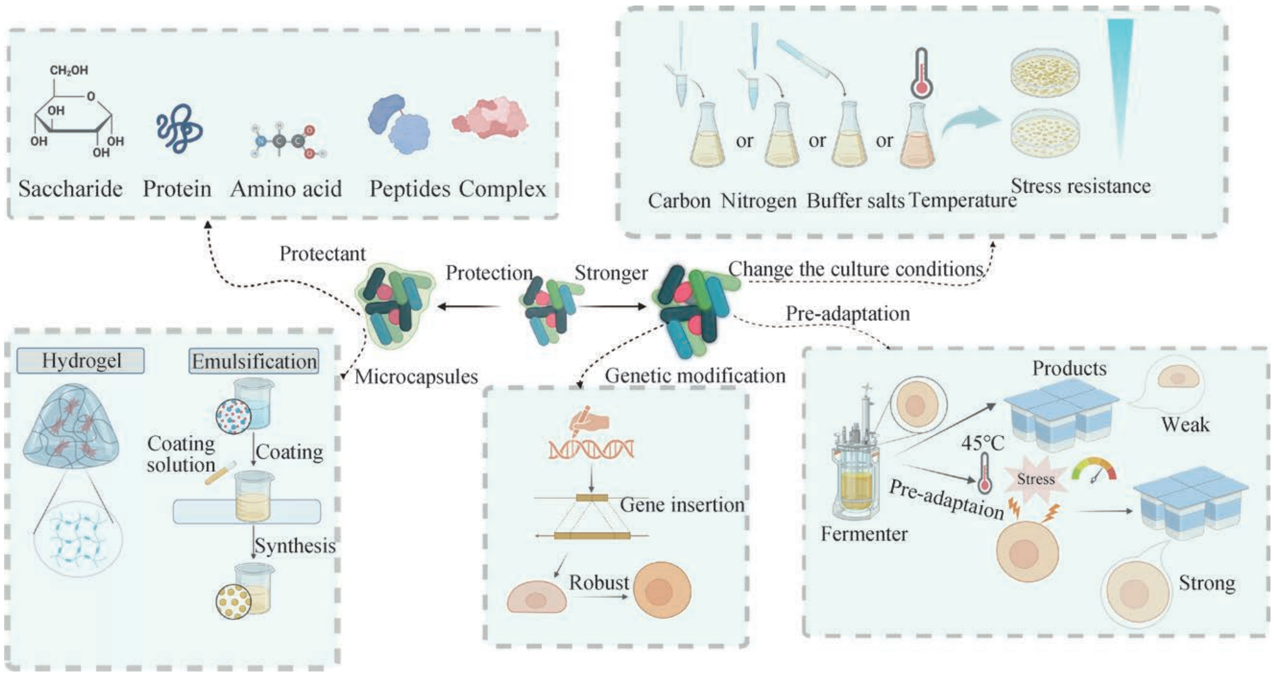

图2 胁迫环境下对LAB的不同保护策略

Figure 2 Different protection strategies for LAB in stressful environments. Created with BioRender.com.

冷冻干燥后的存活率在 83%–85% 之间[64]。蛋白质类也是冻干保护剂的重要一部分，Ying 等[65]发现乳清蛋白的基质缓冲能力可以让 LAB 在极端的 pH 环境中保持活力。除一些常见物质之外，一些氨基酸物质则能够结合细胞内的蛋白质结构中的氨基基团，从而稳定蛋白质的功能[66]，并且能够调节再水化过程中的 Zeta 电位，恢复表面电荷后从而提高对 Caco-2 细胞系的黏附能力，使得菌株更好地存活于胃肠道环境[67]。除使用单一的保护剂之外，研究人员也探究了不同种类保护剂的复配方式。Savedboworn 等[68]通过将大米蛋白和低聚果糖组合作为植物乳杆菌的冻干保护剂，其能够保持细胞表面高度的疏水性，最后冻干后的菌粉与活菌对食源性致病菌的抑制能力无差异。

Zhou 等[69]研究发现，L-谷氨酸钠是对具有促睡眠功能的植物乳杆菌 L-5 的高效冻干保护剂，通过调控丙酮酸生物合成和代谢等过程来提高菌株对真空和冷应激的响应能力，活菌数 6 个月后仍稳定在 4.80×10^{11} CFU/g。

2.1.2 微囊化

微囊化技术是指利用天然或合成的高分子材料作为壁材，将固体、液体等芯材包埋在生物相容性聚合物基质或壳体内的一类技术，微囊化已被证明是保护 LAB 免遭上消化道降解的一项有效手段[70]。有研究报道微胶囊化可保护益生菌免受胃液侵蚀，提高其在胃肠道中的存活率[71]。刘会平等[72]利用海藻酸钠和氯化钙离子交换反应对干酪乳杆菌进行双层包埋，在 2–6 ℃下干燥 36–72 h 后，所得的微胶囊具有较强的耐酸耐胆盐能力与储藏稳定性。除此之外，微囊化也是抵抗加工过程环境胁迫的有效方式。Sun 等[60]通过干燥动力学实验发现，海藻糖-乳清蛋白-支链淀粉凝胶体系具有优异的持水性，这归因于海藻糖降低了水凝胶的储能模量，减弱了蛋白质和多糖之间的疏水相互作用、二硫

键和氢键，从而保证了植物乳杆菌生存的最低水分需求，并增强了其在冷冻干燥过程中的活力。根据 LAB 的菌株特异性设计不同的包埋保护方式，不仅能够提高在消化道和储藏时的稳定性，而且能够达到缓释的目的[73]。Eratte 等[74]将干酪乳杆菌与金枪鱼油共包埋于乳清蛋白分离物——阿拉伯胶络合物中，体外模拟消化发现，由于总 omega-3 脂肪酸释放量的提高，使得微胶囊在通过唾液、胃液之后干酪乳杆菌还有较高的存活率，并提高了黏附在肠壁上的能力。

现有的微囊化的方式仍有许多不足，高成本以及复杂的工艺设计限制了大部分微囊化的工业应用，其次，LAB 微囊化的粒径、形态不能满足所有产品的需求。因此，如何根据菌株的特异性与 LAB 产品应用场景而设计不同的微囊化方法是一个值得深入研究的领域。

2.2 增强菌株抗逆性

除在外部隔离胁迫环境之外，增强菌株本身的抗逆性更为重要。目前相关的研究方法主要可分为改变培养条件、预适应、基因工程三类。图 3 所示为增强菌株抗逆性的相关机制，包含 pH-稳态、能量代谢以及转录和蛋白水平的变化。

2.2.1 改变培养条件

LAB 在增殖、冷冻干燥、消化过程中受到胁迫之后具有复杂且多样的调控机制[2]。LAB 的培养条件包含温度、pH、碳源、氮源、缓冲盐等，研究人员发现冷冻干燥过程中，与改变冻干参数或使用冷冻保护剂相比，改变培养条

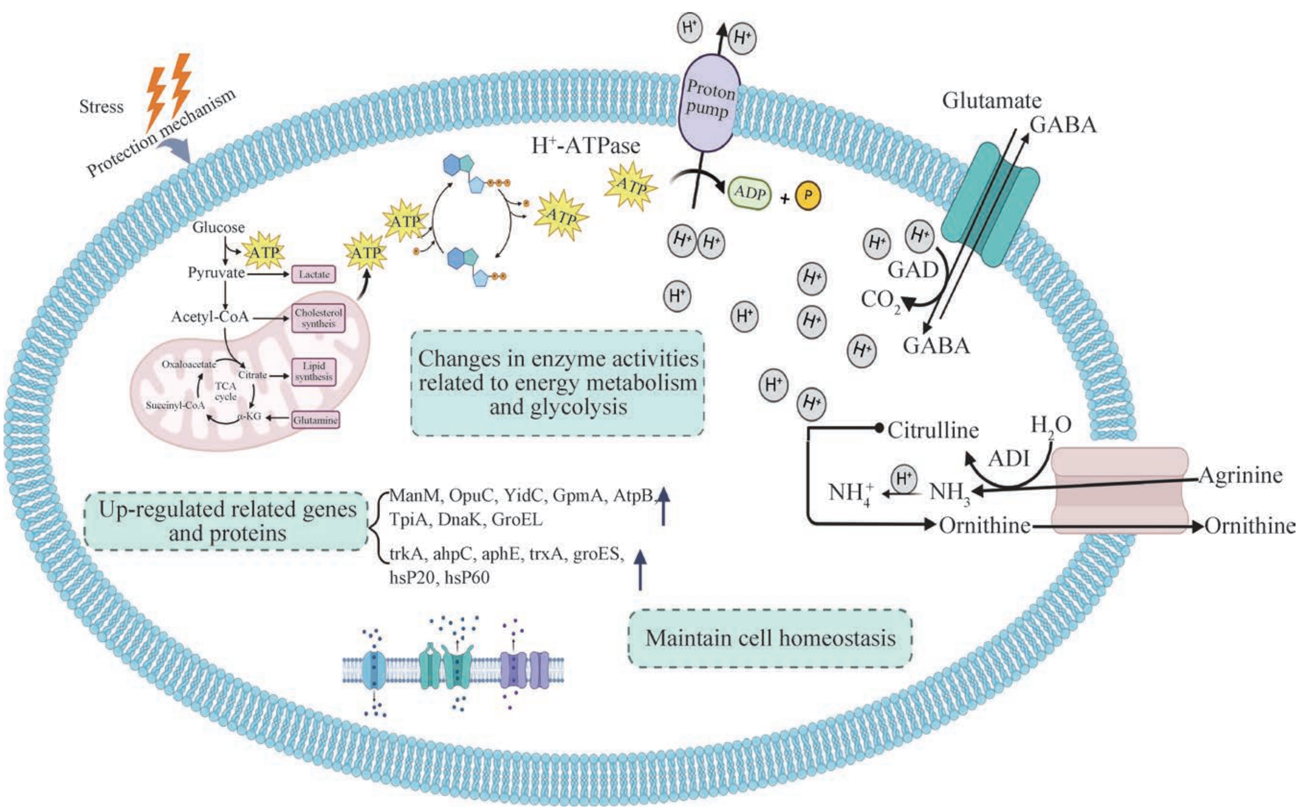

图3 增强LAB菌株抗逆性的相关机制

Figure 3 The mechanisms related to enhancing stress resistance in LAB strains. Created with BioRender.com.

件更具有降低成本和提高效率的优势[75]。Wang 等[76]将嗜酸乳杆菌在 30 °C 或 pH 为 5.0 的条件下培养时，细胞通过改变细胞膜的饱和与不饱和脂肪酸的比例获得了更好的冷冻抗性。在探究碳源成分时，Silva 等[77]发现在保加利亚杆菌的培养基中添加蔗糖后，在增强抗逆性方面比其他碳源更具优势。在 Li 等[78]研究中，保加利亚乳杆菌在含吐温-20 和蔗糖的培养基中增殖时，由于环丙烷脂肪酸含量的显著增加，冷冻干燥后存活率明显提高。除碳源因素外，氮源对 LAB 的细胞活性的影响同样受到关注。Shao 等[79]发现，培养基中若含有酵母提取物反而会降低保加利亚乳杆菌的低温耐受性，探究得出酵母提取物会使菌体生长得更长，加大了低温对细胞的损伤，因此冻干存活率降低。同样地，在包维臣[80]的培养基优化实验中，将氮源酵母浸膏替换成动物蛋白后，菌株存活率更高。

酸胁迫是工业生产和胃肠道消化中遇到的重要生存挑战之一。Broadbent 等[81]在培养基中添加 30 mmol/L 组氨酸后，干酪乳杆菌通过在胞内积累苹果酸和组氨酸使其在酸性条件下的存活率提高了 100 倍。Wu 等[82]也发现低 pH 环境会导致干酪乳杆菌中天冬氨酸和精氨酸的积累，后通过外源添加这 2 种氨基酸的方式使菌株在 pH 3.3 条件下的存活率分别提高了 1.36 倍和 2.10 倍。Wang 等[83]则通过代谢组学发现 pH-稳态机制相关的氨基酸还有赖氨酸、谷氨酸，它们与菌株的黏附能力密切相关。除外源添加氨基酸之外，培养基的酸碱性也是影响 LAB 抗逆性的一项因素。E 等[84]在探究培养基中的 L-谷氨酸冷冻干燥保护机制时，发现酸性环境不仅会上调与细胞膜完整性相关的 *dnaK* 基因和 *secG* 基因的表达，还会上调 *murL*、*murD* 和 *vanY* 基因的表达来增加肽聚糖的合成。E 等[85]还通过调整植物乳杆菌 LIP-1 培养基的缓冲体系，从转录水平分析出缓冲盐中的 K^+ 上调了 *LuxS*/autoinducer-2 (AI-2)群体感应系统中 *luxS* 基因的表达，促进了生物膜形成，从而赋予目标菌株更高的冻干存活率和更好的储藏稳定性。

除了上述常见的培养基成分外，还可以根据菌株的特异性添加其他能增强其抗逆性的成分。然而，培养条件的调整应该以实际生产为靶向，与生产工艺程序设计相结合才更具有现实意义。

2.2.2 预适应

LAB 本身适应压力的能力在抵抗恶劣环境时至关重要。除上述保护策略之外，预适应是将 LAB 暴露于具有挑战性的环境之前在亚致死条件下适应性培养，已被证明可以有效提高其存活率[86]。近年来，报道了多项关于通过温和环境的预先适应来提高 LAB 在各种胁迫条件下的耐受性和活性的工作，在极端压力下，很难通过调节自身的生理代谢来阻止环境因素对菌体的伤害，例如冻干时的低温、人体的消化道环境。表 1 总结了已报道的不同预适应方式提高菌株抗逆性的机制。

2.2.3 基因工程

近年来，通过转录组学或蛋白质组学方法鉴定了许多与应激保护相关的基因/蛋白质。因此，胁迫耐受性的改善也可以通过基因修饰、诱导已有基因的表达或插入相关基因来实现。对关键基因/蛋白质的表达进行基因工程改造，使 LAB 在各种胁迫条件下获得更好的活力是一种潜在的策略[102]。Zhao 等[103]发现重组菌株在酸-乙醇胁迫下，*ctsR* 基因的异源表达使 ROS 积累减少并增强细胞膜完整性，并提高了 Ca^{2+}/Mg^{2+}-ATP 酶和 Na^+/K^+-ATP 酶的活性，首次证明 *ctsR* 基因增强了植物乳杆菌的耐酸乙醇性。Darsonval 等[104]则发现，*ctsR* 基因可以充当温度传感器，使得菌株在 33 °C 以上的生长温度下失活；若抑制酒球菌属中的 *ctsR* 基因表达会

表1　不同预适应方式对LAB的影响

Table 1　Effect of different pretreatment methods on LAB

Methods	Strains	Term	Results	Mechanisms	References
Heat	*Enterococcus faecium*	52 ℃ 15 min	Increased tolerance to H_2O_2, ethanol, acid, and alkaline stresses		[4]
	Tetragenococcus halophilus	45 ℃ 1.5 h	Seven-fold increase in survival in ethanol-stressed environments	Involvement in energy metabolism and upregulation of membrane transporter protein genes	[87]
	Lactobacillus acidophilus	65 ℃ 40 min	Increased stability when stored at 37 ℃ and 42 ℃ for one week		[15]
	Lactobacillus acidophilus	45 ℃ 30 min	Freeze-drying survival rate increased from 39.1% to 56.3%	Changes in key enzymes of glycolysis and Na^+-K^+ ATPase and increased galactose production	[88]
Cold	*Streptococcus thermophilus*	10 ℃ 2 h	Increased tolerance to gastric fluids		[89]
	Lactobacillus delbrueckii ssp. *bulgaricus*	10 ℃ 2 h	Lyophilisation survival rate increased by 16.06%	Expression of two cold shock-induced genes and six heat shock-induced genes were upregulated	[79]
	Lactobacillus kefiranofaciens	20 ℃ 1 h	Inducing cross-stress and increasing resistance to other adversarial environments	Up-regulation of the expression of the molecular chaperones *DnaK* and *GroEl*	[90]
	Lactobacillus helveticus	10 ℃ 2 h		Increased synthesis of cyclopropane fatty acids and enhanced cell membrane fluidity	[91]
	Lactobacillus brevis	−5 ℃ 2 h	Extended shelf life	Production of surface proteins	[92]
Acid	*Lactobacillus casei*	pH 4.5 10 min	100-fold increase in survival at pH 2.5	Intracellular malate and histidine accumulation	[81]
	Lactobacillus plantarum	pH 3.0 1 h	Increased survival rate after 180 days of storage at room temperature		[93]
	Lactobacillus delbrueckii ssp. *bulgaricus*	pH 5.0 MRS	Freeze-drying survival rate increased to 68.3%		[79]
	Lactobacillus plantarum	pH 4.5 2 h	Increased lyophilisation survival	Genes involved in fatty acid synthesis and amino acid metabolism and sugar metabolism were significantly upregulated	[94]
NaCl	*Lactobacillus acidophilus*	0.6 mol/L NaCl		Increased synthesis of surface proteins	[95]
	Lactobacillus plantarum	0.4 mol/L NaCl	Increased lyophilisation survival	Up-regulation of K^+ transporter-related *trkA* gene increases cell membrane unsaturated fatty acid content	[96]

(待续)

(续表 1)

Methods	Strains	Term	Results	Mechanisms	References
	Lactobacillus delbrueckii ssp. *bulgaricus*	0.2 mol/L NaCl		Increased accumulation of the compatible solute glycine betaine	[97]
	Lactobacillus delbrueckii ssp. *bulgaricus*	2% NaCl 2 h	Increased glucose utilisation	Increased activity of glycolytic enzymes	[98]
H_2O_2	*Bifidobacterium animalis*	1.5 mmol/L H_2O_2		Up-regulation of genes encoding thioredoxin systems and divalent cation transporter protein genes	[99]
	Lactobacillus rhamnosus	0.5 mmol/L H_2O_2	Increased survival in sublethal conditions	Enhanced regulation of amino acid metabolism and group sensing pathways	[100]
	Bifidobacterium	210 ppm dissolved oxygen	No loss of viability after 35 days of storage		[101]

导致菌株的可培养性显著下降，突出了 *ctsR* 在酒球菌属应激反应中的关键作用。Watthanasakphuban 等研究利用 pSIP 表达系统成功构建了 2 株能够表达活性氧清除酶的重组菌株，能最大限度地减少细胞中 ROS 的形成，使工业菌株在氧化应激条件下仍能获得较高的细胞密度[105]。这一发现有益于易受氧化应激的 LAB 在工厂高密度发酵中的应用。

从基因层面上挖掘菌株耐受性机制之后可以通过对基因进行修饰、诱导、插入来获得具有抗逆性较好的工程菌株，但同时也要面临和解决基因工程的 LAB 产品所带来的安全隐患。

3 总结与展望

在 LAB 的生产、储藏、消化过程中，环境胁迫是不可避免的。为了充分利用 LAB 在食品工业中作为发酵剂、在医学中作为益生菌、免疫调节剂和活疫苗的潜力，深入了解微生物的复杂防御机制，并根据产品需求设计高活性保护策略，是实现将 LAB 转化为工业上生产可行性产品的基础。本文总结了 LAB 从生产到人体摄入一系列过程所遭受的渗透压、温度、氧、酸、胆盐胁迫等的应激反应，并从外源保护和提高菌株本身的抗逆性两大方面阐述了现有的应激保护策略及其机制。调节机制决定了 LAB 细胞对应激源的敏感性，长期暴露于亚致死剂量的应激源已被证明会在种群水平上引起适应性和微进化的改变。如何根据菌种对不同的应激源进行保护策略的调整对之后 LAB 产品菌种的筛选和改良，以及生产工艺优化都具有重要指导意义。

目前，许多 LAB 菌株抗逆性研究的重点聚焦于如何提高环境胁迫下菌株的活力，而菌株的功能特性在经过保护策略处理之后是否改变常常被忽视，LAB 功能活性的探究是开发益于人体健康产品的关键。鉴于日渐多元的 LAB 应用形式，未来的研究可以着重于 LAB 菌株的保护策略与不同应用产品(如食品)结合后是否仍能发挥原始的功能活性。另外，一项具有实际意义的 LAB 菌株保护策略，应当是既能与工业生产程序设计紧密结合，又能兼顾到工业上成本的经济性，这样才能实现科学成果向实际生产应用的良好转化。

作者利益冲突公开声明

作者声明不存在任何可能会影响本文所报告工作的已知经济利益或个人关系。

参考文献

[1] HE GQ, WU CD, HUANG J, ZHOU RQ. Acid tolerance response of *Tetragenococcus halophilus*: a combined physiological and proteomic analysis[J]. Process Biochemistry, 2016, 51(2): 213-219.

[2] PAPADIMITRIOU K, ALEGRÍA Á, BRON PA, de ANGELIS M, GOBBETTI M, KLEEREBEZEM M, LEMOS JA, LINARES DM, ROSS P, STANTON C, TURRONI F, van SINDEREN D, VARMANEN P, VENTURA M, ZÚÑIGA M, TSAKALIDOU E, KOK J. Stress physiology of lactic acid bacteria[J]. Microbiology and Molecular Biology Reviews, 2016, 80(3): 837-890.

[3] SHARMA A, LAVANIA M, SINGH R, LAL B. Identification and probiotic potential of lactic acid bacteria from camel milk[J]. Saudi Journal of Biological Sciences, 2021, 28(3): 1622-1632.

[4] SHIN Y, KANG CH, KIM W, SO JS. Heat adaptation improved cell viability of probiotic *Enterococcus faecium* HL7 upon various environmental stresses[J]. Probiotics and Antimicrobial Proteins, 2019, 11(2): 618-626.

[5] LI Y, GAO JS, XUE L, SHANG YY, CAI WC, XIE XQ, JIANG T, CHEN HZ, ZHANG JM, WANG J, CHEN MT, DING Y, WU QP. Determination of antiviral mechanism of centenarian gut-derived *Limosilactobacillus fermentum* against norovirus[J]. Frontiers in Nutrition, 2022, 9: 812623.

[6] 吴清平, 陈慧贞, 李滢, 谢新强, 张菊梅, 杨宁, 陈惠元, 代京莎, 陈玲, 刘振杰. 一株高效合成烟酰胺、抗光老化的发酵乳杆菌 XJC60 及其应用: CN113832050B[P]. 2022-10-21.
WU QP, CHEN HZ, LI Y, XIE XQ, ZHANG JM, YANG N, CHEN HY, DAI JS, CHEN L, LIU Z. Lactobacillus fermentum capable of efficiently synthesizing nicotinamide and resisting light aging and application of *Lactobacillus fermentum*: CN113832050B[P]. 2022-10-21 (in Chinese).

[7] 吴清平, 商燕燕, 李滢, 谢新强, 丁郁, 王涓, 薛亮, 陈谋通, 张菊梅, 叶青华, 吴诗, 陈惠元, 吴军林. 一株产溶菌酶并高效拮抗多药耐药幽门螺杆菌的植物乳杆菌LP1Z及其应用: CN114350578B[P]. 2022-05-27.
WU QP, SHANG YY, LI Y, XIE XQ, DING Y, WANG J, XUE L, CHEN MT, ZHANG JM, YE QH, WU S, CHEN HY, WU J. *Lactobacillus plantarum* LP1Z capable of producing lysozyme and efficiently antagonizing multidrug-resistant helicobacter pylori and application of LP1Z: CN114350578B[P]. 2022-05-27 (in Chinese).

[8] WU L, XIE XQ, LI Y, LIANG TT, ZHONG HJ, YANG LS, XI Y, ZHANG JM, DING Y, WU QP. Gut microbiota as an antioxidant system in centenarians associated with high antioxidant activities of gut-resident *Lactobacillus*[J]. NPJ Biofilms and Microbiomes, 2022, 8(1): 102.

[9] YANG LS, XIE XQ, LI Y, WU L, FAN CC, LIANG TT, XI Y, YANG SH, LI HX, ZHANG JM, DING Y, XUE L, CHEN MT, WANG J, WU QP. Evaluation of the cholesterol-lowering mechanism of *Enterococcus faecium* strain 132 and *Lactobacillus paracasei* strain 201 in hypercholesterolemia rats[J]. Nutrients, 2021, 13(6): 1982.

[10] LI LY, LIANG TT, JIANG T, LI Y, YANG LS, WU L, YANG J, DING Y, WANG J, CHEN MT, ZHANG JM, XIE XQ, WU QP. Gut microbiota: candidates for a novel strategy for ameliorating sleep disorders[J]. Critical Reviews in Food Science and Nutrition, 2024, 64(29): 10772-10788.

[11] FIOCCO D, LONGO A, ARENA MP, RUSSO P, SPANO G, CAPOZZI V. How probiotics face food stress: they get by with a little help[J]. Critical Reviews in Food Science and Nutrition, 2020, 60(9): 1552-1580.

[12] DERUNETS AS, SELIMZYANOVA AI, RYKOV SV, KUZNETSOV AE, BEREZINA OV. Strategies to enhance stress tolerance in lactic acid bacteria across diverse stress conditions[J]. World Journal of Microbiology & Biotechnology, 2024, 40(4): 126.

[13] CHEN X, WU JH, YANG FJ, ZHOU M, WANG RB, HUANG JL, RONG YZ, LIU JH, WANG SY. New insight into the mechanism by which antifreeze peptides regulate the physiological function of *Streptococcus thermophilus* subjected to freezing stress[J]. Journal of Advanced Research, 2023, 45: 127-140.

[14] LIU Y, LIU B, LI D, HU YL, ZHAO L, ZHANG M, GE SY, PANG J, LI YX, WANG R, WANG PJ, HUANG YT, HUANG J, BAI J, REN FZ, LI Y. Improved gastric acid resistance and adhesive colonization of probiotics by mucoadhesive and intestinal targeted konjac glucomannan microspheres[J]. Advanced Functional Materials, 2020, 30(35): 2001157.

[15] KULKARNI S, HAQ SF, SAMANT S, SUKUMARAN S. Adaptation of *Lactobacillus acidophilus* to thermal stress yields a thermotolerant variant which also exhibits improved survival at pH 2[J]. Probiotics and Antimicrobial Proteins, 2018, 10(4): 717-727.

[16] PARLINDUNGAN E, JONES OAH. Using metabolomics to understand stress responses in lactic acid bacteria and their applications in the food industry[J]. Metabolomics, 2023, 19(12): 99.

[17] POPOVA-KRUMOVA P, DANOVA S, ATANASOVA N, YANKOV D. Lactic acid production by *Lactiplantibacillus plantarum* AC 11S-kinetics and modeling[J]. Microorganisms, 2024, 12(4): 739.

[18] BUSTOS AY, TARANTO MP, GEREZ CL, AGRIOPOULOU S, SMAOUI S, VARZAKAS T, ENSHASY HAE. Recent advances in the understanding of stress resistance mechanisms in probiotics: relevance for the design of functional food systems[J]. Probiotics and Antimicrobial Proteins, 2024. Doi: 10.1007/s12602-024-10273-9.

[19] BELENKY P, COLLINS JJ. Microbiology. Antioxidant strategies to tolerate antibiotics[J]. Science, 2011, 334 (6058): 915-916.

[20] 王学良, 韩雪, 王海娟, 井雪萍, 刘采云, 周艳. 乳酸菌在

各种胁迫下的应激反应研究进展[J]. 食品工业科技, 2015, 36(6): 365-369.
WANG XL, HAN X, WANG HJ, JING XP, LIU CY, ZHOU Y. Studying progress of *Lactobacillus*'s responses in a variety of stress[J]. Science and Technology of Food Industry, 2015, 36(6): 365-369 (in Chinese).

[21] ZHU ZM, YANG JH, YANG PS, WU ZM, ZHANG J, DU GC. Enhanced acid-stress tolerance in *Lactococcus lactis* NZ9000 by overexpression of ABC transporters[J]. Microbial Cell Factories, 2019, 18(1): 136.

[22] JAN G, LEVERRIER P, PICHEREAU V, BOYAVAL P. Changes in protein synthesis and morphology during acid adaptation of *Propionibacterium freudenreichii*[J]. Applied and Environmental Microbiology, 2001, 67(5): 2029-2036.

[23] LEE MY, KANG MJ, CHA S, KIM TR, PARK YS. Acid tolerance responses and their mechanisms in *Lactiplantibacillus plantarum* LM1001[J]. Food Science and Biotechnology, 2024, 33(9): 2213-2222.

[24] BOMMASAMUDRAM J, KUMAR P, KAPUR S, SHARMA D, DEVAPPA S. Development of thermotolerant lactobacilli cultures with improved probiotic properties using adaptive laboratory evolution method[J]. Probiotics and Antimicrobial Proteins, 2023, 15(4): 832-843.

[25] KÖNIG P, AVERHOFF B, MÜLLER V. A first response to osmostress in *Acinetobacter baumannii*: transient accumulation of K^+ and its replacement by compatible solutes[J]. Environmental Microbiology Reports, 2020, 12(4): 419-423.

[26] BROWN AD, SIMPSON JR. Water relations of sugar-tolerant yeasts: the role of intracellular polyols[J]. Journal of General Microbiology, 1972, 72(3): 589-591.

[27] BERGENHOLTZ ÅS, WESSMAN P, WUTTKE A, HÅKANSSON S. A case study on stress preconditioning of a *Lactobacillus* strain prior to freeze-drying[J]. Cryobiology, 2012, 64(3): 152-159.

[28] 高薇, 韩雪, 张兰威. 乳酸菌渗透胁迫相关相容性溶质及其转运机制研究进展[J]. 微生物学通报, 2013, 40(11): 2097-2106.
GAO W, HAN X, ZHANG LW. Research advances of the osmotic stress-related compatible solutes of lactic acid bacteria and it's transport mechanism[J]. Microbiology China, 2013, 40(11): 2097-2106 (in Chinese).

[29] BRUGER EL, HYING ZT, SINGLA D, MÁRQUEZ REYES NL, PANDEY SK, PATEL JS, BAZURTO JV. Enhanced catabolism of *Glycine* betaine and derivatives provides improved osmotic stress protection in *Methylorubrum extorquens* PA1[J]. Applied and Environmental Microbiology, 2024, 90(7): e0031024.

[30] WOOD JM, BREMER E, CSONKA LN, KRAEMER R, POOLMAN B, van der HEIDE T, SMITH LT. Osmosensing and osmoregulatory compatible solute accumulation by bacteria[J]. Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology, 2001, 130(3): 437-460.

[31] ROBERTS MF. Organic compatible solutes of halotolerant and halophilic microorganisms[J]. Saline Systems, 2005, 1: 5.

[32] SLEATOR RD, HILL C. Bacterial osmoadaptation: the role of osmolytes in bacterial stress and virulence[J]. FEMS Microbiology Reviews, 2002, 26(1): 49-71.

[33] GALLO M, PASSANNANTI F, COLUCCI CANTE R, NIGRO F, SCHIATTARELLA P, ZAPPULLA S, BUDELLI A, NIGRO R. Lactic fermentation of cereals aqueous mixture of oat and rice flours with and without glucose addition[J]. Heliyon, 2020, 6(9): e04920.

[34] BISUTTI IL, STEPHAN D. Influence of fermentation temperature and duration on survival and biocontrol efficacy of *Pseudomonas fluorescens* Pf153 freeze-dried cells[J]. Journal of Applied Microbiology, 2020, 128(1): 232-241.

[35] JOFRÉ A, AYMERICH T, GARRIGA M. Impact of different cryoprotectants on the survival of freeze-dried *Lactobacillus rhamnosus* and *Lactobacillus casei*/*paracasei* during long-term storage[J]. Beneficial Microbes, 2015, 6(3): 381-386.

[36] GAGNETEN M, PASSOT S, CENARD S, GHORBAL S, SCHEBOR C, FONSECA F. Mechanistic study of the differences in lactic acid bacteria resistance to freeze- or spray-drying and storage[J]. Applied Microbiology and Biotechnology, 2024, 108(1): 361.

[37] LIU B, FU N, WOO MW, CHEN XD. Heat stability of *Lactobacillus rhamnosus* GG and its cellular membrane during droplet drying and heat treatment[J]. Food Research International, 2018, 112: 56-65.

[38] 公丕民. 保加利亚乳杆菌喷雾干燥过程中损伤机制及保护方法研究[D]. 哈尔滨: 哈尔滨工业大学博士学位论文, 2019.
GONG PM. Study on damage mechanism and protection method of *Lactobacillus bulgaricus* during spray drying[D]. Harbin: Doctoral Dissertation of Harbin Institute of Technology, 2019 (in Chinese).

[39] SHU GW, WANG Z, CHEN L, WAN HC, CHEN H. Characterization of freeze-dried *Lactobacillus acidophilus* in goat milk powder and tablet: optimization of the composite cryoprotectants and evaluation of storage stability at different temperature[J]. LWT, 2018, 90: 70-76.

[40] 谢建松, 杨占国, 安铎. 玻璃化转变对食品干燥贮藏的影响[J]. 粮食流通技术, 2012(3): 34-36.
XIE JS, YANG ZG, AN D. Effects on food drying and storage of glass transition[J]. Grain Distribution Technology, 2012(3): 34-36 (in Chinese).

[41] KATHIRIYA MR, VEKARIYA YV, HATI S. Understanding the probiotic bacterial responses against various stresses in food matrix and gastrointestinal tract: a review[J]. Probiotics and Antimicrobial Proteins, 2023, 15(4): 1032-1048.

[42] IMLAY JA. Cellular defenses against superoxide and hydrogen peroxide[J]. Annual Review of Biochemistry, 2008, 77: 755-776.

[43] GIBSON CM, MALLETT TC, CLAIBORNE A, CAPARON MG. Contribution of NADH oxidase to aerobic metabolism of *Streptococcus pyogenes*[J]. Journal of Bacteriology, 2000, 182(2): 448-455.

[44] FENG T, WANG J. Oxidative stress tolerance and antioxidant capacity of lactic acid bacteria as probiotic: a

systematic review[J]. Gut Microbes, 2020, 12(1): 1801944.
[45] LI Y, HUGENHOLTZ J, ABEE T, MOLENAAR D. Glutathione protects *Lactococcus lactis* against oxidative stress[J]. Applied and Environmental Microbiology, 2003, 69(10): 5739-5745.
[46] JÄNSCH A, KORAKLI M, VOGEL RF, GÄNZLE MG. Glutathione reductase from *Lactobacillus sanfranciscensis* DSM20451T: contribution to oxygen tolerance and thiol exchange reactions in wheat sourdoughs[J]. Applied and Environmental Microbiology, 2007, 73(14): 4469-4476.
[47] LIN QJ, LIN SY, FAN ZT, LIU J, YE DC, GUO PT. A review of the mechanisms of bacterial colonization of the mammal gut[J]. Microorganisms, 2024, 12(5): 1026.
[48] GUAN NZ, LIU L. Microbial response to acid stress: mechanisms and applications[J]. Applied Microbiology and Biotechnology, 2020, 104(1): 51-65.
[49] YANG X, HANG XM, ZHANG M, LIU XL, YANG H. Relationship between acid tolerance and cell membrane in *Bifidobacterium*, revealed by comparative analysis of acid-resistant derivatives and their parental strains grown in medium with and without Tween-80[J]. Applied Microbiology and Biotechnology, 2015, 99(12): 5227-5236.
[50] WEI YX, GAO J, LIU DB, LI Y, LIU WL. Adaptational changes in physiological and transcriptional responses of *Bifidobacterium longum* involved in acid stress resistance after successive batch cultures[J]. Microbial Cell Factories, 2019, 18(1): 156.
[51] WANG YY, XU HR, ZHOU XQ, CHEN WD, ZHOU HP. Dysregulated bile acid homeostasis: unveiling its role in metabolic diseases[J]. Medical Review, 2024, 4(4): 262-283.
[52] HAY AJ, ZHU J. Chapter two in sickness and in health the relationships between bacteria and bile in the human gut[J]. Advances in Applied Microbiology, 2016, 96: 43-64.
[53] 陈春萌. 消化应激对乳酸菌黏附能力的影响[D]. 扬州: 扬州大学硕士学位论文, 2021.
CHEN CM. Effect of digestive stress on adhesion of lactic acid bacteria[D]. Yangzhou: Master's Thesis of Yangzhou University, 2021 (in Chinese).
[54] KEBOUCHI M, GALIA W, GENAY M, SOLIGOT C, LECOMTE X, AWUSSI AA, PERRIN C, ROUX E, DARY-MOUROT A, le ROUX Y. Implication of sortase-dependent proteins of *Streptococcus thermophilus* in adhesion to human intestinal epithelial cell lines and bile salt tolerance[J]. Applied Microbiology and Biotechnology, 2016, 100(8): 3667-3679.
[55] SÁNCHEZ B, CHAMPOMIER-VERGÈS MC, ANGLADE P, BARAIGE F, de LOS REYES-GAVILÁN CG, MARGOLLES A, ZAGOREC M. Proteomic analysis of global changes in protein expression during bile salt exposure of *Bifidobacterium longum* NCIMB 8809[J]. Journal of Bacteriology, 2005, 187(16): 5799-5808.
[56] 乌日娜. 益生菌 *Lactobacillus casei* Zhang 蛋白质组学研究[D]. 呼和浩特: 内蒙古农业大学博士学位论文, 2009.
WU RN. Protein omics of probiotic *Lactobacillus casei* Zhang[D]. Hohhot: Doctoral Dissertation of Inner Mongolia Agricultural University, 2009 (in Chinese).
[57] BUSTOS AY, SAAVEDRA L, de VALDEZ GF, RAYA RR, TARANTO MP. Relationship between bile salt hydrolase activity, changes in the internal pH and tolerance to bile acids in lactic acid bacteria[J]. Biotechnology Letters, 2012, 34(8): 1511-1518.
[58] GRILL JP, CAYUELA C, ANTOINE JM, SCHNEIDER F. Isolation and characterization of a *Lactobacillus amylovorus* mutant depleted in conjugated bile salt hydrolase activity: relation between activity and bile salt resistance[J]. Journal of Applied Microbiology, 2000, 89(4): 553-563.
[59] KHODER G, AL-MENHALI AA, AL-YASSIR F, KARAM SM. Potential role of probiotics in the management of gastric ulcer[J]. Experimental and Therapeutic Medicine, 2016, 12(1): 3-17.
[60] SUN HY, ZHANG MH, LIU YK, WANG Y, CHEN YY, GUAN WY, LI X, WANG YH. Improved viability of *Lactobacillus plantarum* embedded in whey protein concentrate/pullulan/trehalose hydrogel during freeze drying[J]. Carbohydrate Polymers, 2021, 260: 117843.
[61] 吴家琳, 李滢, 刘振杰, 陈谋通, 吴清平. 益生菌冷冻干燥高活性保护机制研究进展[J]. 微生物学报, 2024, 64(5): 1402-1416.
WU JL, LI Y, LIU ZJ, CHEN MT, WU QP. Research progress in the mechanism of freeze-drying in protecting the high vitality of probiotics[J]. Acta Microbiologica Sinica, 2024, 64(5): 1402-1416 (in Chinese).
[62] KUMAR A, CINCOTTI A, APARICIO S. A theoretical study on trehalose + water mixtures for dry preservation purposes[J]. Molecules, 2020, 25(6): 1435.
[63] GE ST, HAN JR, SUN QY, YE ZD, ZHOU QQ, LI P, GU Q. Optimization of cryoprotectants for improving the freeze-dried survival rate of potential probiotic *Lactococcus lactis* ZFM559 and evaluation of its storage stability[J]. LWT, 2024, 198: 116052.
[64] STEFANELLO RF, NABESHIMA EH, IAMANAKA BT, LUDWIG A, FRIES LLM, BERNARDI AO, COPETTI MV. Survival and stability of *Lactobacillus fermentum* and *Wickerhamomyces anomalus* strains upon lyophilisation with different cryoprotectant agents[J]. Food Research International, 2019, 115: 90-94.
[65] YING DY, PHOON MC, SANGUANSRI L, WEERAKKODY R, BURGAR I, AUGUSTIN MA. Microencapsulated *Lactobacillus rhamnosus* GG powders: relationship of powder physical properties to probiotic survival during storage[J]. Journal of Food Science, 2010, 75(9): E588-E595.
[66] WANG L, HE MY, WU T, YANG KY, WANG YL, ZHANG Y, GU YC, DENG KW. Screening of the freeze-drying protective agent for high-quality milk beer yeast (*Kluyveromyces marxianus*) and optimization of freeze-drying process conditions[J]. Journal of Food Processing and Preservation, 2021, 45(12): e16016.
[67] ARELLANO K, PARK H, KIM B, YEO S, JO H, KIM JH, JI Y, HOLZAPFEL WH. Improving the viability of freeze-dried probiotics using a lysine-based rehydration

mixture[J]. Microbiology and Biotechnology Letters, 2021, 49(2): 157-166.
[68] SAVEDBOWORN W, TEAWSOMBOONKIT K, SURICHAY S, RIANSA-NGAWONG W, RITTISAK S, CHAROEN R, PHATTAYAKORN K. Impact of protectants on the storage stability of freeze-dried probiotic *Lactobacillus plantarum*[J]. Food Science and Biotechnology, 2018, 28(3): 795-805.
[69] ZHOU R, WU YW, LI Y, LI LY, WU JL, XIE XQ, HUANG HS, GAO H, WU L, ZHAO H, CHEN MT, WU QP. Sodium l-glutamate improves the lyophilization survival rate of *Lactiplantibacillus plantarum* L5 by regulating cellular pyruvate[J]. Food Bioscience, 2024, 59: 104189.
[70] COOK MT, TZORTZIS G, CHARALAMPOPOULOS D, KHUTORYANSKIY VV. Microencapsulation of a synbiotic into PLGA/alginate multiparticulate gels[J]. International Journal of Pharmaceutics, 2014, 466(1/2): 400-408.
[71] DODOO CC, WANG J, BASIT AW, STAPLETON P, GAISFORD S. Targeted delivery of probiotics to enhance gastrointestinal stability and intestinal colonisation[J]. International Journal of Pharmaceutics, 2017, 530(1/2): 224-229.
[72] 刘会平, 王潆雪, 胡赞扬. 一种干酪乳杆菌微胶囊的制备方法: CN101724622A[P]. 2010-06-09.
LIU HP, WANG YX, HU ZY. A type of preparation method of Lactobacillus casei microcapsules. CN101724622A[P]. 2010-06-09 (in Chinese).
[73] FAYED B, ABOOD A, EL-SAYED HS, HASHEM AM, MEHANNA NSH. A synbiotic multiparticulate microcapsule for enhancing inulin intestinal release and *Bifidobacterium* gastro-intestinal survivability[J]. Carbohydrate Polymers, 2018, 193: 137-143.
[74] ERATTE D, DOWLING K, BARROW CJ, ADHIKARI BP. *In-vitro* digestion of probiotic bacteria and omega-3 oil co-microencapsulated in whey protein isolate-gum Arabic complex coacervates[J]. Food Chemistry, 2017, 227: 129-136.
[75] E JJ, WANG PX, WANG RX, ZHANG QL, WANG JG. Effects of L-cysteine on the freeze-drying survival rate of *Lactiplantibacillus plantarum* LIP-1[J]. Food Science of Animal Products, 2023, 9240044.
[76] WANG Y, CORRIEU G, BÉAL C. Fermentation pH and temperature influence the cryotolerance of *Lactobacillus acidophilus* RD758[J]. Journal of Dairy Science, 2005, 88(1): 21-29.
[77] SILVA J, CARVALHO AS, PEREIRA H, TEIXEIRA P, GIBBS PA. Induction of stress tolerance in *Lactobacillus delbrueckii* ssp. *bulgaricus* by the addition of sucrose to the growth medium[J]. Journal of Dairy Research, 2004, 71(1): 121-125.
[78] LI C, LIU LB, LIU N. Effects of carbon sources and lipids on freeze-drying survival of *Lactobacillus bulgaricus* in growth media[J]. Annals of Microbiology, 2012, 62(3): 949-956.
[79] SHAO YY, GAO SR, GUO HL, ZHANG HP. Influence of culture conditions and preconditioning on survival of *Lactobacillus delbrueckii* subspecies *bulgaricus* ND02 during lyophilization[J]. Journal of Dairy Science, 2014, 97(3): 1270-1280.
[80] 包维臣. 德氏乳杆菌保加利亚亚种ND02高密度培养及冷冻保护的研究[D]. 呼和浩特: 内蒙古农业大学硕士学位论文, 2012.
BAO WC. Study on high density culture and cryopreservation of *Lactobacillus delbrueckii* subsp. bulgaricus ND02[D]. Hohhot: Master's Thesis of Inner Mongolia Agricultural University, 2012 (in Chinese).
[81] BROADBENT JR, LARSEN RL, DEIBEL V, STEELE JL. Physiological and transcriptional response of *Lactobacillus casei* ATCC 334 to acid stress[J]. Journal of Bacteriology, 2010, 192(9): 2445-2458.
[82] WU CD, ZHANG J, CHEN W, WANG M, DU GC, CHEN J. A combined physiological and proteomic approach to reveal lactic-acid-induced alterations in *Lactobacillus casei* Zhang and its mutant with enhanced lactic acid tolerance[J]. Applied Microbiology and Biotechnology, 2012, 93(2): 707-722.
[83] WANG WW, HE JY, PAN DD, WU Z, GUO YX, ZENG XQ, LIAN LW. Metabolomics analysis of *Lactobacillus plantarum* ATCC 14917 adhesion activity under initial acid and alkali stress[J]. PLoS One, 2018, 13(5): e0196231.
[84] E JJ, ZHANG JY, MA RZ, CHEN ZC, YAO CQ, WANG RX, ZHANG QL, YANG Y, LI J, WANG JG. Study of the internal mechanism of L-glutamate for improving the survival rate of *Lactiplantibacillus plantarum* LIP-1 after freeze-drying[J]. Innovative Food Science & Emerging Technologies, 2023, 84: 103253.
[85] E JJ, MA RZ, CHEN ZC, YAO CQ, WANG RX, ZHANG QL, HE ZB, SUN RY, WANG JG. Improving the freeze-drying survival rate of *Lactobacillus plantarum* LIP-1 by increasing biofilm formation based on adjusting the composition of buffer salts in medium[J]. Food Chemistry, 2021, 338: 128134.
[86] ZHANG CC, LU JY, YANG D, CHEN X, HUANG YJ, GU RX. Stress influenced the aerotolerance of *Lactobacillus rhamnosus* hsryfm 1301[J]. Biotechnology Letters, 2018, 40(4): 729-735.
[87] YANG H, YAO SJ, ZHANG M, WU CD. Heat adaptation induced cross protection against ethanol stress in *Tetragenococcus halophilus*: physiological characteristics and proteomic analysis[J]. Frontiers in Microbiology, 2021, 12: 686672.
[88] ZHEN N, ZENG XQ, WANG HJ, YU J, PAN DD, WU Z, GUO YX. Effects of heat shock treatment on the survival rate of *Lactobacillus acidophilus* after freeze-drying[J]. Food Research International, 2020, 136: 109507.
[89] FANG SH, LAI YJ, CHOU CC. The susceptibility of *Streptococcus thermophilus* 14085 to organic acid, simulated gastric juice, bile salt and disinfectant as influenced by cold shock treatment[J]. Food Microbiology, 2013, 33(1): 55-60.
[90] CHEN MJ, TANG HY, CHIANG ML. Effects of heat, cold, acid and bile salt adaptations on the stress tolerance and protein expression of kefir-isolated probiotic *Lactobacillus kefiranofaciens* M1[J]. Food Microbiology, 2017, 66: 20-27.

[91] MONTANARI C, SADO KAMDEM SL, SERRAZANETTI DI, ETOA FX, GUERZONI ME. Synthesis of cyclopropane fatty acids in *Lactobacillus helveticus* and *Lactobacillus sanfranciscensis* and their cellular fatty acids changes following short term acid and cold stresses[J]. Food Microbiology, 2010, 27(4): 493-502.

[92] CHOI IS, KO SH, KIM HM, CHUN HH, LEE KH, YANG JE, JEONG S, PARK HW. Shelf-life extension of freeze-dried *Lactobacillus brevis* WiKim0069 using supercooling pretreatment[J]. LWT, 2019, 112: 108230.

[93] BARBOSA J, BORGES S, TEIXEIRA P. Influence of sub-lethal stresses on the survival of lactic acid bacteria after spray-drying in orange juice[J]. Food Microbiology, 2015, 52: 77-83.

[94] HUANG RH, PAN MF, WAN CX, SHAH NP, TAO XY, WEI H. Physiological and transcriptional responses and cross protection of *Lactobacillus plantarum* ZDY2013 under acid stress[J]. Journal of Dairy Science, 2016, 99(2): 1002-1010.

[95] PALOMINO MM, WAEHNER PM, FINA MARTIN J, OJEDA P, MALONE L, SÁNCHEZ RIVAS C, PRADO ACOSTA M, ALLIEVI MC, RUZAL SM. Influence of osmotic stress on the profile and gene expression of surface layer proteins in *Lactobacillus acidophilus* ATCC 4356[J]. Applied Microbiology and Biotechnology, 2016, 100(19): 8475-8484.

[96] WANG RX, SUN RY, YANG Y, JINGJING E, YAO CQ, ZHANG QL, CHEN ZC, MA RZ, LI J, ZHANG JY, WANG JG. Effects of salt stress on the freeze-drying survival rate of *Lactiplantibacillus plantarum* LIP-1[J]. Food Microbiology, 2022, 105: 104009.

[97] HAN X, WU HY, YU P, ZHANG LJ, ZHAO SN, ZHANG LW. Glycine betaine transport conditions of *Lactobacillus delbrueckii* subsp. *bulgaricus* in salt induced hyperosmotic stress[J]. International Dairy Journal, 2018, 86: 21-26.

[98] LI C, SUN JW, QI XX, LIU LB. NaCl stress impact on the key enzymes in glycolysis from *Lactobacillus bulgaricus* during freeze-drying[J]. Brazilian Journal of Microbiology, 2015, 46(4): 1193-1199.

[99] ZHANG JL, WANG SB, ZENG Z, QIN YX, LI PL. The complete genome sequence of *Bifidobacterium animalis* subsp. *lactis* 01 and its integral components of antioxidant defense system[J]. 3 Biotech, 2019, 9(10): 352.

[100] ZHANG CC, GUI Y, CHEN X, CHEN DW, GUAN CR, YIN BX, PAN ZM, GU RX. Transcriptional homogenization of *Lactobacillus rhamnosus* hsryfm 1301 under heat stress and oxidative stress[J]. Applied Microbiology and Biotechnology, 2020, 104(6): 2611-2621.

[101] TALWALKAR A, KAILASAPATHY K. Oxidative stress adaptation of probiotic bacteria[J]. Milchwissenschaft-Milk Science International, 2004, 59: 140-143.

[102] YANG H, HE MW, WU CD. Cross protection of lactic acid bacteria during environmental stresses: stress responses and underlying mechanisms[J]. LWT, 2021, 144: 111203.

[103] ZHAO HY, YUAN L, HU K, LIU LX, PENG S, LI H, WANG H. Heterologous expression of ctsR from *Oenococcus oeni* enhances the acid-ethanol resistance of *Lactobacillus plantarum*[J]. FEMS Microbiology Letters, 2019, 366(15): fnz192.

[104] DARSONVAL M, JULLIAT F, MSADEK T, ALEXANDRE H, GRANDVALET C. CtsR, the master regulator of stress-response in *Oenococcus oeni*, is a heat sensor interacting with ClpL1[J]. Frontiers in Microbiology, 2018, 9: 3135.

[105] WATTHANASAKPHUBAN N, SRILA P, PINMANEE P, SOMPINIT K, RATTANAPORN K, PETERBAUER C. Development of high cell density *Limosilactobacillus reuteri* KUB-AC5 for cell factory using oxidative stress reduction approach[J]. Microbial Cell Factories, 2023, 22(1): 86.