

微生物源食品保鲜剂的研究进展

徐畅, 于基成, 刘秋

(大连民族大学 生命科学学院, 辽宁 大连 116600)

摘要: **目的** 介绍近5年微生物源食品保鲜剂的研究进展, 为研究高效、无毒、天然的食品保鲜剂提供理论和方法依据。 **方法** 综述常见的微生物源食品保鲜剂, 包括细菌源保鲜剂、真菌源保鲜剂和微生物代谢产物保鲜剂(乳酸链球菌素、 ϵ -聚赖氨酸、溶菌酶和纳他霉素)。简要说明其抑菌机理和存在的问题。 **结果** 微生物源保鲜剂可以通过竞争营养, 诱导系统抗性和产生活性代谢产物等方式抑制多种致病菌的生长繁殖, 降低果蔬病害的发生率, 保持食品良好的感官品质和理化特性, 有效延长食品货架期。 **结论** 微生物源保鲜剂为食品保鲜贮藏提供了新途径。其抑菌机理和潜在毒性需进一步明确, 如何提高微生物源保鲜剂抗不良环境的稳定性还有待进一步研究。

关键词: 微生物源; 保鲜剂; 活性代谢产物; 食品保鲜

中图分类号: TS205.9 **文献标识码:** A **文章编号:** 1001-3563(2021)13-0009-12

DOI: 10.19554/j.cnki.1001-3563.2021.13.002

Research Progress on Microbial Source Food Preservatives

XU Chang, YU Ji-cheng, LIU Qiu

(College of Life Science, Dalian Minzu University, Dalian, 116600, China)

ABSTRACT: The paper aims to introduce the advances of microbial source food preservatives in recent five years and to provide theoretical foundation and novel methods for the development of efficient, non-toxic and natural food preservatives. Several microbial food preservatives including bacterial preservative, fungal preservative and microbial active metabolites (Nisin, ϵ -polylysine, lysozyme and natamycin) were summarized, and their antibacterial mechanism and existing problems were briefly introduced. Microbial source food preservatives generally could generally inhibit the growth of pathogenic bacteria, reduce the incidence of fruit and vegetable diseases, maintain good sensory quality and physical-chemical characteristics by nutrition competition, inducing system resistance and producing active metabolites etc., thereby extending food shelf-life. Microbial preservatives provided a novel pathway for food preservation and storage. But the antibacterial mechanism and potential toxicity should be further clarified, and the studies to improve the stability of microbial preservatives against adverse environment are needed.

KEY WORDS: microbial source; preservative; active metabolite; food preservation

食品和新鲜果蔬及其制品在贮藏、运输和销售的过程中常因氧化、生理代谢、微生物污染等原因腐败变质。为防止其腐烂变质, 减少营养损失, 各种食品

保鲜剂被广泛开发, 并应用于食品中。虽然传统的化学保鲜剂效果较好, 但可能引起环境污染, 甚至食品中的残留会导致健康风险。寻求安全、高效、环境友

收稿日期: 2021-03-27

基金项目: 国家自然科学基金(31770006); 大连民族大学学科团队项目(2021)

作者简介: 徐畅(1995—), 女, 大连民族大学硕士生, 主攻食品质量与安全控制。

通信作者: 于基成(1968—), 男, 博士, 大连民族大学教授, 主要研究方向为食品质量与安全控制。

好的食品保鲜剂尤为重要。相较于化学保鲜剂,天然保鲜剂具有来源广,安全性高,对食品的营养和感官特性影响较小等特点^[1]。天然保鲜剂按其来源主要分为动物源、植物源和微生物源保鲜剂^[2],其中微生物源保鲜剂具有抗菌能力强、微生物生长周期短、易改良、易培养等优点,被食品工业广泛关注^[1]。文中主要介绍几种微生物和微生物活性代谢产物在食品保鲜领域的应用现状,为开发新型环境友好、高效、安全的保鲜剂提供借鉴。

1 微生物源的保鲜剂

新鲜果蔬在贮藏、运输等过程中可能受到机械创伤,使其易受到微生物侵染而腐烂,造成严重的食物浪费和经济损失。近年来,许多学者相继筛选出对采后致病菌有抑制作用的拮抗微生物,主要有木霉属^[3]、芽孢杆菌属^[4]、假单胞菌属^[5]和酵母属^[6]等,其已经被证明有作为生防剂用于果蔬采后贮藏保鲜的潜力^[7]。虽然微生物拮抗剂的活性已被广泛证实,但大多数微生物拮抗剂对采后病害的作用机理尚不明确。一般认为微生物源的保鲜剂涉及寄主、病原菌、生防菌和环境之间的复杂相互作用,其中包括竞争空间、碳源和铁,产生裂解酶和抗菌物质,以及诱导植物抗性过程^[8]。

1.1 细菌源保鲜剂

芽孢杆菌属(*Bacillus* spp.)在自然界中分布广泛,对恶劣的物理化学环境(如热、紫外线和有机溶剂)的抵抗力强^[4]。芽孢杆菌对人类和动物无毒无害,可以增加植物的系统抗性^[9-10],被认为是传统化学杀菌剂的生物安全替代品。到目前为止,已有将枯草芽孢杆菌(*Bacillus subtilis*)^[11]、淀粉芽孢杆菌(*Bacillus cereus*)^[12]、短短芽孢杆菌(*Brevibacillus brevis*)^[13]等用来防治果蔬采后病害的报道。例如,ZHANG等^[9]研究表明,枯草芽孢杆菌 JK-14 菌株对桃子采后致病性最强的细链格孢菌(*Alternaria tenuis*)和灰霉病菌(*Botrytis cinerea*)的生长有明显的拮抗作用,接种 5 d 后对 2 种致病菌的抑制率分别为 81.32%和 83.45%,显著降低了黑星病和灰霉病的发病率。Fan等^[14]初步解释了枯草芽孢杆菌(*Bacillus Subtilis* 9407)防治苹果环腐病机理,发现 *Bacillus Subtilis* 9407 的抗真菌活性与 ppsB 基因有关。ppsB 基因负责合成的化合物芬芥素可有效抑制致病菌生长。车建美等^[13]制备了一种性状稳定、活菌含量高的短短芽孢杆菌保鲜菌剂,其可显著提高枇杷果实的好果率,降低果实的质量损失率,保持果实可溶性固形物的含量。杜婵娟等^[15]通过紫外线-亚硝酸钠复合诱变的方法得到短短芽孢杆菌 Bb5911,其抗菌率提高了 112.41%。该菌株与低浓度(10.42 μg/mL)咪鲜胺复配使用对

香蕉炭疽病的防治效果相当于高浓度(333.33 μg/mL)咪鲜胺的防治效果,可达到减少农药使用量的目的。淀粉芽孢杆菌是芽孢杆菌属另一重要的生防菌,经淀粉芽孢杆菌处理后的荔枝保持了较高的可溶性固形物、可溶性总糖和维生素 C 含量,并保持了较高的抗病相关酶(苯丙氨酸解氨酶、几丁质酶和 3-葡聚糖酶)活性^[16]。淀粉芽孢杆菌还被用于防治葡萄采后灰霉病^[12]、枇杷采后炭疽病^[17]、果汁中酵母菌的污染^[18]。

乳酸菌(*Lactic acid bacteria*)是另一重要应用的细菌,其一方面通过合成乳酸,降低 pH 值的方式来抑制食源性致病菌的生长,另一方面通过代谢产生细菌素、H₂O₂ 和羟基脂肪酸等抗菌化合物实现生物保鲜^[19]。植物乳杆菌(*Lactobacillus plantarum*) DSM-20174 菌株对采后病原菌黄曲霉(*Aspergillus flavus*)、尖头炭疽菌(*Colletotrichum acutatum*)、胶孢炭疽菌(*Colletotrichum gloeosporioides*)和燕麦镰刀菌(*Fusarium avenaceum*)孢子萌发的抑制率可达 89.62%~97.61%^[20]。RUSSO^[21]研究发现,高浓度的植物乳杆菌和发酵乳杆菌(*Lactobacillus fermentum*)可成功应用于鲜切菠萝的保鲜加工,在维持鲜切菠萝的营养价值和感官特性的同时,对相关食源性致病菌单增李斯特菌(*Listeria monocytogenes*)和大肠杆菌(*E.coli* O₁₅₇:H₇)均有拮抗作用。SHARMA^[22]从牛乳和人乳中分离出乳酸菌,发现其对蜡样芽孢杆菌(*Bacillus cereus*)、伤寒沙门氏菌(*Salmonella enterica* serovar Typhi)、福氏志贺氏菌(*Shigella flexneri*)、铜绿假单胞菌(*Pseudomonas aeruginosa*)有一定的抑制作用,可作为抗菌剂应用于食品保鲜。干酪乳杆菌 YZU01 可通过分泌胞外代谢产物来降解棒曲霉素,处理 36 h 可完全降解苹果汁或梨汁中的棒曲霉素^[23]。鼠李糖乳杆菌可使鲜切甜椒货架期从 8 d 延长至 12 d^[24]。在酸奶发酵过程中添加鼠李糖乳杆菌可抑制大肠杆菌、金黄色葡萄球菌等多种食源性致病菌^[25]。乳酸菌还通常用于防治作物在种植期间的病害,例如 JACOBO 等^[7]证明了植物乳杆菌 LPLUV10 可以抑制尖孢镰刀菌(*Fusarium oxysporum*)的生长,对番茄植株具有较强的保护作用。植物乳杆菌 PM411, TC54, TC92 可有效定殖于苹果和梨植株上,可作为微生物杀虫剂的有效成分用于防治欧文氏菌(*Erwinia Amylovora*)引起的火疫病^[26]。

荧光假单胞菌(*Pseudomonas fluorescens*)可通过产生挥发性抑菌化合物^[5],产生嗜铁素、氢氰酸和 2,4-二乙酰基间苯三酚^[27]等抗生素,以及诱导寄主系统抗性^[28]来抑制真菌病原体。荧光假单胞菌被广泛用于采后保鲜,尤其是霉菌引起的青霉病^[29]、灰霉病^[30]。与对照组相比,苹果经荧光假单胞菌 1-112 和 2-28 菌液浸泡后,其青霉病的发病率分别降低了 68%和 88%^[31]。除了病原体威胁,褐变也是降低苹果

价值的重要原因之一。ISABEL 等^[32]将禾谷假单胞菌与气调保鲜技术配合使用,在 10℃下保存 7 d 后,单增李斯特菌菌落总数减少 2.5 lg CFU/g,沙门氏菌菌落总数减少了 3.3 lg CFU/g。绿针假单胞菌 (*Pseudomonas chlororaphis* SPS-41) 可以产生 3-甲基-1-丁醇、苯乙醇和 2-甲基-1-丁醇等挥发性有机化合物,其可以抑制菌丝的生长和孢子的繁殖,对几种植物病原菌均表现出广泛的抗真菌活性^[33]。ZHANG 等^[33]认为绿针假单胞菌 SPS-41 是防治甘薯黑腐病的一种潜在的生物熏蒸剂。目前在食品保鲜中应用的主要细菌源保鲜剂见表 1。

1.2 真菌源保鲜剂

与细菌相比,酵母菌的基因更加稳定。到目前为止,已有大量的拮抗酵母菌被分离和筛选。据文献报道,酵母菌是抑制草莓灰霉病的有效菌^[6,50]。CHEN 等^[51]筛选出 24 株对草莓灰霉病菌有拮抗作用的酵母菌,研究发现,其中 6 个菌株可以产生扩散性抑制化合物,所有菌株都可以产生挥发性抑制化合物,其中部分菌株可以通过分泌水解酶(几丁质酶、蛋白酶和纤维素酶)来水解真菌细胞,从而起到拮抗作用。多数拮抗酵母菌是直接从水果表面分离出来的,其在植株的叶子、根部和土壤中广泛存在。从枇杷果实上分离筛选的季也蒙毕赤酵母 Y35-1 菌株可有效抑制炭疽病原真菌胶孢炭疽菌 (*Colletotrichum gloeosporioides*) 孢子的萌发,以及菌丝的扩展和生长。枇杷经酵母 Y35-1 处理后,其腐烂率可控制在 2%~3% (贮藏 20 d)^[52]。从柑橘表面分离的梅奇酵母 (*Metschnikowia* sp.FL02) 可在 7 d 内完全抑制柑橘绿霉病的发生^[53]。从柑橘类水果中分离的发酵毕赤酵母 (*Pichia fermentans* 27) 在整个柠檬采收期表现出高度拮抗性,其保护效果甚至优于以嗜油假丝酵母为基础的商业化产品^[54]。肉类和香肠的调味和贮藏过程

中易受霉菌的污染,利用拮抗酵母菌可有效抑制肉类食品中产毒真菌的繁殖^[55]。扣囊复膜孢酵母 (*Saccharomycopsis fibuligera*) 和汉森酵母 (*Debaryomyces hansenii*) 可在肉制品表面定殖,在保持其原有感官特性的同时,可以作为生防剂抑制产赭曲霉毒素中赭曲霉和青霉的生长^[56]。同时发现,汉森酵母可抑制黄曲霉素生物合成相关基因 aflR 和 aflS 的表达,并通过竞争空间和营养,抑制有害真菌的生长繁殖,进而抑制了干发酵、干腌渍肉制品表面寄生曲霉的生存^[57-58]。嗜油假丝酵母^[59-60]、红酵母^[61-62]等生防酵母也是作为微生物源食品保鲜剂的极佳候选者。

木霉 (*Trichoderma* spp.) 常见于土壤和植物根际,其可在植物组织全生育期扩展定殖,并通过重寄生、抗生素作用,以及诱导植物局部和系统的抗性反应^[3],抑制病原菌的侵染和存活。木霉菌对病原菌的拮抗作用在生物防治领域得到了广泛的研究,已经被用来保鲜香蕉^[63]、甘薯^[64]、山药^[65]等多种食品。例如,绿色木霉 (*Trichoderma viride*)、哈茨木霉 (*Trichoderma Harzianum*) 和康宁木霉 (*Trichoderma kningii*) 的混合作用显著降低了香蕉冠腐病的发病率,比化学杀菌剂——多菌灵具有更好的防治效果^[66]。DANIA^[67]对接种绿色木霉、哈茨木霉、钩状木霉 (*Trichoderma hamatum*)、康宁木霉 4 个月后甘薯的腐烂程度进行了评价,发现 4 种木霉菌共产生 24 种代谢产物,其中哈茨木霉产生的代谢产物最多,其使尖孢镰刀菌、立枯丝核菌 (*Rhizoctonia solani*) 等甘薯采后致病菌的菌丝生长量减少了 54.6%~77.3%。哈茨木霉培养液参杂复合薄荷精油可使已经感染 1×10⁷ CFU/mL 镰刀菌 (*fusarium* sp.) 的甜菜根继续保存 7 d^[68]。虽然大多数微生物剂在受控环境条件下表现良好,但实践中受到多种因素影响,应用效果不佳。有研究者将微生物拮抗剂与有机或无机载体结合,增强其对病原菌的

表 1 细菌源保鲜剂在食品保鲜中的应用
Tab.1 Application of bacteria-derived antiskating agent in food preservation

菌属	中文名称	拉丁文名称	食品种类
芽孢杆菌属	淀粉芽孢杆菌	<i>Bacillus amyloliquefaciens</i>	葡萄 ^[12] 、荔枝 ^[16] 、枇杷 ^[17] 、橙汁 ^[18]
	枯草芽孢杆菌	<i>Bacillus subtilis</i>	桃子 ^[9] 、草莓 ^[11] 、苹果 ^[14] 、葡萄 ^[34]
	短短芽孢杆菌	<i>Brevibacillus brevis</i>	枇杷 ^[13] 、香蕉 ^[15] 、梨 ^[35]
乳酸菌属	植物乳杆菌	<i>Lactobacillus plantarum</i>	鸡肉 ^[36] 、莲藕 ^[37] 、草莓 ^[38]
	发酵乳杆菌	<i>Lactobacillus fermentum</i>	菠萝 ^[21] 、面包 ^[39]
	干酪乳杆菌	<i>Lactobacillus casei</i>	果汁 ^[23] 、酸奶 ^[40]
	鼠李糖乳杆菌	<i>Lactobacillus rhamnosus</i>	甜椒 ^[24] 、酸奶 ^[25] 、婴儿食品 ^[41] 、梨 ^[42]
假单胞菌属	荧光假单胞菌	<i>Pseudomonas fluorescens</i>	苹果 ^[31] 、柑橘 ^[43] 、番茄 ^[44]
	禾谷假单胞菌	<i>Pseudomonas graminis</i>	苹果 ^[32] 、梨 ^[45] 、甜瓜 ^[46]
	绿针假单胞菌	<i>Pseudomonas chlororaphis</i>	甘薯 ^[33] 、可可豆 ^[47] 、番茄 ^[48] 、辣椒 ^[49]

实际生物防治效果^[69]。NIKOO 等^[69]利用哈茨木霉、棘胞木霉 (*Trichoderma asperellum*) 和黄蓝状菌 (*Talaromyces flavus*) 与 3 种载体 (泥炭、米糠和滑石) 开发了多种生物制剂, 研究发现, 所有生物制剂均显著增加了温室内甜菜健康幼苗的数量。此外, 木霉常被用于防治玉米茎腐病^[70]、辣椒赤霉病^[70]、水稻纹枯病^[71]、马铃薯晚疫病^[72]等。目前在食品保鲜中应用的主要真菌源保鲜剂见表 2。

2 微生物代谢产物食品保鲜剂

微生物能够产生多种代谢产物, 包括乳酸链球菌素 (Nisin)^[76]、ε-聚赖氨酸 (Natamycin)^[77]、溶菌酶 (Lysozyme)^[78]等, 因其具有抑菌、无毒, 以及不影响食品本身风味口感的功能特点, 被广泛用于食品保鲜^[1]。

2.1 乳酸链球菌素

乳酸链球菌素 (Nisin) 又称乳链菌肽, 是一种由乳酸链球菌产生的活性抗菌肽, 可以有效地抑制引起食品腐败的革兰氏阳性菌^[79]。目前关于 Nisin 的抑菌机理可分为 2 个: 与菌体细胞膜上的 Lipid II (细菌萜醇-焦磷酸-N-乙酰胞壁酸-五肽-N-乙酰葡萄糖胺) 分子结合, 形成 Nisin-lipid II 复合物, 在细胞膜上形成穿孔通道, 使细胞内物质外流; Lipid II 分子是细胞壁合成的重要中间体, 其与 Lipid II 结合后, 阻碍细胞壁的正常合成, 抑制细胞生长^[80]。Nisin 作为食品防腐剂已获得粮食及农业组织/世界卫生组织 (FAO/WHO) 的许可。目前, Nisin 已广泛用于乳制品^[81]、肉制品^[76]、罐藏食品^[82]等领域的食品保鲜。

由于 Nisin 能抑制嗜热性细菌的生长繁殖, 因此经 Nisin 处理的乳制品避免了因高温加热加工而出现的不良风味和营养损失^[82]等问题。Nisin 在中性 pH

条件下缺乏稳定性, 且易与脂肪球相互作用^[81], 使其在乳制品中的应用受限。研究者曾采用玉米醇溶蛋白包埋 Nisin, 研究发现, 其可以显著增强对新鲜奶酪中单增李斯特菌的抗菌效果^[83]。另外, Nisin 的使用方式对其抗菌性有显著影响, SOUZA 等^[76]研究发现, 喷雾法相较于浸渍法更有利于抗菌剂在猪肉表面的分散, 可完全抑制乳酸菌的生长。在应用中也发现, 肉类食品中胰蛋白酶能酶解抑制 Nisin 的活性^[89]。为改善 Nisin 在肉制品中的活性, TANZINA 等^[84]利用海藻酸钠和纤维素纳米晶将 Nisin 微胶囊化, 使用含有不同浓度 Nisin 的胶囊保鲜火腿片 28 d, 结果表明, 单增李斯特菌的菌数比游离 Nisin 组减少了 1.50~3.04 lg CFU/g。DAN 等^[85]认为, Nisin 与大豆蛋白或蛋清蛋白复合使用比单独使用的保鲜效果更好, 这 2 种蛋白中的胰蛋白酶抑制剂能抑制外源胰蛋白酶活性, 从而保护 Nisin 活性, 从根本上解决 Nisin 在应用中对蛋白酶敏感的缺点。

相比革兰氏阳性菌, 革兰氏阴性菌通常对 Nisin 表现出抗药性。有研究发现, 某些螯合剂 (如 EDTA)^[86]可破坏细胞膜的稳定性, 使革兰氏阴性细菌对 Nisin 敏感。LIANG 等^[86]认为, Nisin 和 EDTA 联合使用对副溶血性弧菌和荧光假单胞菌有一定的抑菌作用, 可代替亚硫酸盐应用于海鲜保鲜中。

2.2 ε-聚赖氨酸

ε-聚赖氨酸 (ε-polylysine, ε-PL) 是放线菌代谢产生的具有明显抑菌功能的碱性氨基酸聚合物, 其由 25~35 个 L-赖氨酸通过 α-ε 酰胺键依次连接而成^[87]。ε-聚赖氨酸的抑菌机理主要表现在以下 2 个方面: 破坏菌体细胞膜和细胞壁的完整性^[88]; 破坏蛋白合成系统, 抑制微生物生长代谢^[89]。ε-PL 作为一种新型食品防腐剂, 因其抑菌谱广、抑制浓度小、水溶性及热稳定性好等特点广受关注。

表 2 真菌源保鲜剂在食品保鲜中的应用
Tab.2 Application of fungus-derived antistaling agent in food preservation

菌属	中文名称	拉丁文名称	食品种类
酵母菌属	发酵毕赤酵母	<i>Pichia fermentans</i>	柠檬 ^[54]
	季也蒙毕赤酵母	<i>Pichia guilliermondii</i>	枇杷 ^[52] 、桃 ^[73] 、苹果 ^[74]
	扣囊复膜孢酵母	<i>Saccharomycopsis fibuligera</i>	肉制品 ^[56] 、番石榴 ^[75]
	汉森酵母	<i>Debaryomyces hansenii</i>	肉制品 ^[56—58]
	嗜油假丝酵母	<i>Candida oleophilic</i>	猕猴桃 ^[59] 、梨 ^[60]
	红酵母	<i>Rhodotorula</i>	柑橘 ^[61] 、草莓 ^[62]
木霉属	哈茨木霉	<i>Trichoderma Harzianum</i>	香蕉 ^[66] 、甘薯 ^[67] 、甜菜 ^[68]
	绿色木霉	<i>Trichoderma viride</i>	香蕉 ^[66] 、甘薯 ^[67]
	棘胞木霉	<i>Trichoderma asperellum</i>	山药 ^[65]

2004 年,美国食品药品监督管理局(FDA)^[76]正式批准 ϵ -PL 为食品防腐剂,我国于 2014 年批准 ϵ -PL 可作为新型食品添加剂应用于食品防腐。目前, ϵ -聚赖氨酸已应用于奶酪^[90]、肉制品^[91]等食品保鲜中。有研究表明,在米饭中添加 100~150 mg/kg 的 ϵ -PL 时,可有效降低菌落总数,在 30 °C 下可以保存 8 d^[92]。经 ϵ -PL 处理过的冷鲜猪肉的菌落总数、挥发性盐基氮(TVB-N)值和高铁肌红蛋白(MetMb)含量会显著降低^[93]。在 4 °C 和 25 °C 下, ϵ -PL 对乳酪中单增李斯特菌有显著的抑制作用^[90]。此外, ϵ -PL 的活性受 pH 和其他离子的影响较大^[94],常将 ϵ -PL 与其他物质联合使用来提高抑菌活性和生物利用度。 ϵ -PL/乳清蛋白配合物可以将酱鸭中大肠杆菌数控制在 5.8 lg CFU/g 以内(贮藏 7 d),只喷洒 ϵ -PL 的实验组大肠杆菌数为 6.5 lg CFU/g^[95]。以壳聚糖-海藻酸钠纳米粒子作为载体的 ϵ -PL 抗菌活性是游离 ϵ -PL 的 3 倍^[96]。 ϵ -PL 与 N,O-羧甲基壳聚糖形成的聚电解质复合物可以克服带正电的 ϵ -PL 与食品中带负电的成分相互作用,避免产生不必要的沉淀和活性丧失,从而进一步延长商品的货架期^[94]。

2.3 溶菌酶

溶菌酶(lysozyme, LZ)又称 N-乙酰胞壁质聚糖水解酶,具有分解细菌细胞壁的作用。溶菌酶主要通过切断细胞壁肽聚糖中的 N-乙酰胞壁酸(NAG)和 N-乙酰葡萄糖胺(NAM)之间的 β -1,4 糖苷键,导致细胞壁破裂,从而达到杀菌的目的^[97-98]。溶菌酶已被欧盟食品添加剂标准(E1105)认定为具有抑菌、溶菌和杀菌活性的功能因子,我国允许将其添加到婴儿奶粉中来增强婴儿的抵抗力^[97]。

溶菌酶除作为食品添加剂外,研究者也尝试将其用于果蔬保鲜。LIU 等^[99]认为,0.5 g/L 溶菌酶会对苹果起到较好的保鲜效果。XU 等^[100]将溶菌酶与 1-甲基环丙烯(1-MCP)联合用于猕猴桃的采后保鲜,二者联用显著抑制了猕猴桃在贮藏过程中的呼吸速率和乙烯的产生,延缓了果实腐烂率、质量损失率、细菌总数的增加,提高了果实的硬度、抗坏血酸含量和抗氧化酶活性。含 7 g/L 溶菌酶的胶原涂膜对鲑鱼片挥发性盐基氮和微生物的抑制效果较好^[101]。高浓度的溶菌酶会产生负面感官评价,使用溶菌酶作为保鲜剂时要选择合适的浓度。

细菌脂多糖(LPS)层、蛋白质和磷脂是革兰氏阴性菌表面的主要成分,由于溶菌酶进入细胞壁的多聚糖层会受到外层脂多糖的阻碍,所以溶菌酶对革兰氏阴性菌几乎无作用^[102]。对溶菌酶进行化学修饰^[102]、吸附^[103]和偶联^[104]来扩展其抑菌谱成为主要研究方向。利用纳米粒子^[103,105]、多糖^[106]等固定溶菌酶是获得活性高、抑菌谱广的溶菌酶的有效途径之一。例如溶菌酶-甲壳素纳米晶体系更容易破坏细菌

细胞,酶活性是游离溶菌酶的 1.5 倍^[103]。JIANG 等^[103]研究发现,溶菌酶-甲壳素纳米晶对革兰氏阴性菌的抗菌效果反而优于革兰氏阳性菌。溶菌酶-N-琥珀酰壳聚糖(LSZ-NSC)活性比游离溶菌酶活性提高了 256%,并且 NSC-LSZ 能有效延长草莓货架期 3 d^[78]。庞莉等^[106]用阿魏酸作为缩合剂,使溶菌酶赖氨酸残基上的 ϵ -氨基共价结合在阿魏酸的羧基上,形成一定程度的阿魏酸修饰酶。虽然修饰酶的活性略有下降,但抑菌谱得到扩展,对革兰氏阴性菌的抑菌作用增强。

2.4 纳他霉素

纳他霉素(Natamycin)是链霉菌的一种次级代谢产物。纳他霉素依靠其内酯环结构,与真菌细胞膜上的甾醇化合物作用,导致细胞膜通透性损伤,物质渗出,菌体死亡。纳他霉素能有效地抑制霉菌、酵母菌等真菌的生长,并阻止丝状真菌中黄曲霉毒素的形成,其对细菌和病毒的作用不显著^[107-108]。纳他霉素在食品工业中被视为天然食品防腐剂,0.5~6 mg/kg 的纳他霉素即可抑制绝大多数的霉菌^[109],目前已广泛用于防止饮料^[110]、乳制品^[111]、水果^[112]、水产品^[113]、肉制品^[114]等多种食品的霉菌污染。

ZHAO 等^[4]利用了纳他霉素对细菌无作用性这一特点,使用纳他霉素协助淀粉芽孢杆菌防治青椒的采后软腐病。纳他霉素显著提高了淀粉芽孢杆菌对胡萝卜欧文氏菌(*Erwinia carotovora subsp.*)的竞争地位。与单独使用淀粉芽孢杆菌处理相比,1 g/L 纳他霉素可使淀粉芽孢杆菌的菌落总数增加 115.8%,胡萝卜欧文氏菌的菌落总数减少 92.1%。姜爱丽等^[115]证明了 20 mg/L 的纳他霉素溶液即可有效控制草莓腐烂,保持较高的花色苷含量,同时也发现纳他霉素在前期会对果实的亮度和酸含量产生负影响。纳他霉素可以提高桑椹贮藏期过氧化氢酶(CAT)、超氧化物歧化酶(SOD)和过氧化物酶(POD)的活性,能有效地保持桑椹的采后品质^[116]。纳他霉素除在果蔬保鲜上被广泛研究,也有研究者将其应用于其他食品(如饮料、乳酪等)。

软饮料因其碳氮比高和 pH 值(3.5)低,易受到醋酸菌、乳酸菌和酵母菌的侵染,饮料中常用的防腐剂苯甲酸易与抗坏血酸形成苯,苯在饮料中累积会影响人体健康^[117]。JULIANO 等^[110]将纳他霉素和 Nisin 代替苯甲酸和山梨酸应用于柠檬味饮料,发现纳他霉素和 Nisin 可维持柠檬味软饮料的感官品质和微生物数量,并显著降低饮料中苯的浓度。将纳他霉素活化的面粉薄膜作为乳酪的包装材料,可使乳酪在保存 14 d 后仍没有霉菌^[118]。

由于纳他霉素的环状化学结构对温度、紫外光、pH 等较为敏感,抑菌性能易受到外界环境的影响^[118],因此研究者将其包封在大分子材料中来避免不利因

素。例如,HAO等^[119]将壳聚糖作为纳他霉素的载体,制备了壳聚糖/纳他霉素涂膜用于保鲜樱桃,可有效抑制了黑霉菌、毛霉菌的侵袭。CLARA等^[120]将纳他霉素保存于异丙基丙烯酸酰胺水凝胶中,在酸性条件下,含有纳他霉素水凝胶的多糖膜的抗菌效率高于不含纳他霉素的多糖膜和含有游离纳他霉素的多糖膜。

3 复合保鲜剂

天然保鲜剂因其具有来源广、安全、成本低等优点而被广泛关注。由于单一保鲜剂不能有效地抑制和杀灭所有的病原菌,因此开发复合保鲜剂成为目前研究的热点。根据栅栏技术的原理,将不同种类的保鲜剂联合使用,克服了单一保鲜剂抑菌谱窄或者需要提高浓度才能起抑菌效果的缺陷,可以进一步提高食品的保鲜效果,延长货架期^[121]。目前,微生物源保鲜剂经常与植物源、动物源保鲜剂等混合使用,以追求更显著的保鲜效果。

有研究发现,虽然含溶菌酶的脂质体可以有效抑制单增李斯特菌的生长,但不能抑制肠炎沙门氏菌,而共包埋 Nisin/溶菌酶混合物的脂质体对这 2 种细菌都有抑制作用^[122]。HWAN 等^[123]将葡萄籽提取物、肉桂醛和 Nisin 组成的复合天然防腐剂用于贮藏牛肉,可有效地保持牛肉色泽,减缓脂肪氧化和蛋白质降解进程,同时食源性病原菌的菌落总数降低了约 1~2 lg CFU/g。经壳聚糖、溶菌酶和茶多酚复合制得的保鲜剂能有效地抑制池沼公鱼在冷藏期间的脂肪氧化,减缓 pH 值和 TVB-N 含量的升高,复合保鲜剂处理使池沼公鱼的货架期从原来的 6 d 延长至 13~15 d^[124]。菊粉、壳聚糖、茶多酚、Nisin 复合保鲜剂可使冷鲜猪肉贮藏 10 d 后仍保持一定的新鲜度^[125]。1-MCP 结合纳他霉素可以降低猕猴桃果实货架期间的代谢水平,有效维持猕猴桃的后熟品质^[126]。

4 微生物源保鲜剂研究的问题和展望

虽然微生物源保鲜剂有巨大的市场开发潜力,但要真正将其推向市场,规模化应用于各类食品的防腐保鲜中还要面临很多挑战。首先,微生物源食品保鲜剂指微生物菌体或微生物活性代谢产物,影响其活性的因素较多,食品成分和外界环境的改变都会造成菌种的死亡和保鲜能力的下降。如何维持微生物源保鲜剂的稳定性应是研究的重点。其次,大多数细菌源、真菌源菌体保鲜剂通过刺伤果实形成伤口来探究其保鲜效果,在商业应用中需要在果实表面达到生防效果,提高菌体在果蔬表面的定殖能力,明确寄主-病原菌-保鲜剂作用模式,以寻求最佳的保鲜效果。再次,目前对微生物源保鲜剂的毒性和抑菌机理的研究尚浅,其可能对人体存在潜在的毒性和安全风险,研

究者应在探讨机理的同时,关注其安全性和风险度。此外,微生物发酵、代谢产物提纯等工艺条件复杂,相较于化学保鲜剂,其具有产量低、不易获取等缺点,这进一步限制了其工业化生产。

基于微生物源保鲜剂现存的问题提出 2 点展望:进行食品毒理学试验,明确不同微生物保鲜剂的使用范围和用量,以及对人体健康的影响;开发新型保鲜剂,运用基因工程技术进行菌株改良,或结合其他物理化学保鲜技术来提高微生物及其代谢产物对不良环境的耐受力,以寻求保鲜效果和利益的最大化。随着人们对食品保鲜剂安全性的高度重视,开发绿色、安全、高效的微生物源保鲜剂的前景必然广阔。

5 结语

随着消费者食品安全意识的不断增强,天然食品保鲜剂的开发应用逐渐成为人们研究的热点。相较于化学保鲜剂,微生物源保鲜剂具有来源广、安全性高和适用性广等优点,其一般通过竞争营养空间、产生挥发性抑制化合物、破坏细胞结构等方式有效防控致病菌的生长,降低果蔬采后病害发病率,较好维持食品感官品质和营养价值,从而延长食品货架期。虽然大量研究表明,微生物源保鲜剂有巨大的市场潜力,但真正实现商业化的产品较少,仍存在活性不稳定,抑菌机理和毒性不明确等诸多问题。微生物源保鲜剂在食品保鲜贮藏领域的发展是机遇和挑战并存的。随着研究的深入,应综合考虑不同保鲜剂的优缺点,依靠基因工程改良菌株,或结合其他保鲜技术来弥补微生物源保鲜剂存在的缺陷。

参考文献:

- [1] GALVEZ A, ABRIQUEL H, BENOMAR N, et al. Microbial Antagonists to Food-Borne Pathogens and Biocontrol[J]. Curr Opin Biotechnol, 2010, 21(2): 142—148.
- [2] 朱亚, 宋纪霖. 天然复合保鲜剂对冷鲜肉感官品质和理化指标的影响[J]. 陕西农业科学, 2018, 64(10): 87—90.
ZHU Ya, SONG Ji-lin. Effects of Natural Compound Preservatives on Sensory Quality and Physicochemical Indexes of Chilled Meat[J]. Shaanxi Journal of Agricultural Sciences, 2018, 64(10): 87—90.
- [3] KUMAR G, MAHARSHI A, PATEL J, et al. Trichoderma: A Potential Fungal Antagonist to Control Plant Diseases[J]. SATSA Mukhapatra-Annual Technical Issue, 2017, 21: 206—218.
- [4] ZHAO Yan-cun, ODHAMBO B O, QIU Jing-ping, et al. Potential of Natamycin in Enhancing the Antagonistic Activity of Bacillus Amyloliquefaciens BGP20

- against Post-Harvest Bacterial Soft Rot of Green Pepper[J]. *Biocontrol Science and Technology*, 2016, 26(3): 402—413.
- [5] KAI M, EFFMERT U, BERG G, et al. Volatiles of Bacterial Antagonists Inhibit Mycelial Growth of the Plant Pathogen *Rhizoctonia Solani*[J]. *Arch Microbiol*, 2007, 187(5): 351—360.
- [6] HUANG R, LI G Q, ZHANG J, et al. Control of Post-harvest Botrytis Fruit Rot of Strawberry by Volatile Organic Compounds of *Candida Intermedia*[J]. *Phytopathology*, 2011, 101(7): 859—869.
- [7] LÓPEZ-SEIJAS J, GARCÍA-FRAGA B, DA SILVA A F, et al. Wine Lactic Acid Bacteria with Antimicrobial Activity as Potential Biocontrol Agents against *Fusarium Oxysporum* F Sp *Lycopersici*[J]. *Agronomy*, 2019, 10(1): 1—12.
- [8] NUNES C A. Biological Control of Postharvest Diseases of Fruit[J]. *European Journal of Plant Pathology*, 2011, 133(1): 181—196.
- [9] ZHANG Shu-wu, ZHENG Qi, XU Bing-liang, et al. Identification of the Fungal Pathogens of Postharvest Disease on Peach Fruits and the Control Mechanisms of *Bacillus Subtilis* Jk-14[J]. *Toxins*, 2019, 11(6): 1—15.
- [10] LASTOCHKINA O, SEIFIKALHOR M, ALINIAEIFARD S, et al. *Bacillus* Spp: Efficient Biotic Strategy to Control Postharvest Diseases of Fruits and Vegetables[J]. *Plants*, 2019, 8(97): 1—24.
- [11] OREGEL-ZAMUDIO E, ANGOA-PÉREZ M V, OYOQUE-SALCEDO G, et al. Effect of Candelilla Wax Edible Coatings Combined with Biocontrol Bacteria on Strawberry Quality During the Shelf-Life[J]. *Scientia Horticulturae*, 2017, 214: 273—279.
- [12] ZHOU Qing-xin, FU Mao-run, XU Min-hui, et al. Application of Antagonist *Bacillus Amyloliquefaciens* NCPSJ7 against *Botrytis Cinerea* in Postharvest Red Globe Grapes[J]. *Food Science & Nutrition*, 2020, 8(3): 1499—1508.
- [13] 车建美, 郑雪芳, 刘波, 等. 短短芽孢杆菌 FJAT-0809-GLX 菌剂的制备及其对枇杷保鲜效果的研究[J]. *保鲜与加工*, 2011, 11(5): 6—9.
- CHE Jian-mei, ZHENG Xue-fang, LIU Bo, et al. Preparation of *Brevibacillus Brevis* FJAT-0809-GLX Agent and Study on Its Effect on Loquats (*Eriobotrya Japonica*)[J]. *Storage and Process*, 2011, 11(5): 6—9.
- [14] FAN Hai-yan, RU Jin-jiang, ZHANG Yuan-yuan, et al. Fengycin Produced by *Bacillus Subtilis* 9407 Plays a Major Role in the Biocontrol of Apple Ring Rot Disease[J]. *Microbiol Res*, 2017, 199: 89—97.
- [15] 杜婵娟, 付岗, 潘连富, 等. 短短芽孢杆菌 Bb5911 的复合诱变及其对香蕉炭疽病的防治作用[J]. *中国生物防治学报*, 2016, 32(6): 767—774.
- DU Chan-juan, FU Gang, Pan Lian-fu, et al. Compound Mutation of *Brevibacillus Brevis* Strain Bb5 911 and Its Control Effect on Banana Anthracnose[J]. *Chinese Journal of Biological Control*, 2016, 32(6): 767—774.
- [16] WU Yi-jing, LIN He-tong, LIN Yi-fen, et al. Effects of Biocontrol Bacteria *Bacillus Amyloliquefaciens* Ly-1 Culture Broth on Quality Attributes and Storability of Harvested Litchi Fruit[J]. *Postharvest Biology and Technology*, 2017, 132: 81—87.
- [17] YAN Fen, LI Chun-hua, YE Xiao-ling, et al. Antifungal Activity of Lipopeptides from *Bacillus Amyloliquefaciens* Mg3 Against *Colletotrichum Gloeosporioides* in Loquat Fruits[J]. *Biological Control*, 2020, 146: 104281.
- [18] SHI Ju-ran, ZHU Xiao-yu, LU Ying-jian, et al. Improving Iturin A Production of *Bacillus Amyloliquefaciens* by Genome Shuffling and Its Inhibition against *Saccharomyces Cerevisiae* in Orange Juice[J]. *Front Microbiol*, 2018, 9: 2683.
- [19] ABDELHAMID A G, EL-DOUGDOUG N K. Controlling Foodborne Pathogens with Natural Antimicrobials by Biological Control and Antivirulence Strategies[J]. *Heliyon*, 2020, 6(9): e05020.
- [20] ZIVKOVIC S, STOSIC S, RISTIC D, et al. Antagonistic Potential of *Lactobacillus Plantarum* Against Some Postharvest Pathogenic Fungi[J]. *Zbornik Matice Srpske Za Prirodne Nauke*, 2019, 136: 79—88.
- [21] RUSSO P, DE CHIARA M L, VERNILE A, et al. Fresh-Cut Pineapple as A New Carrier of Probiotic Lactic Acid Bacteria[J]. *BioMed Research International*, 2014, 2014: 309183.
- [22] SHARMA C, SINGH B P, THAKUR N, et al. Antibacterial Effects of *Lactobacillus* Isolates of Curd and Human Milk Origin against Food-Borne and Human Pathogens[J]. *3 Biotech*, 2017, 7(1): 1—9.
- [23] ZHENG Xiang-feng, WEI Wan-ning, RAO Sheng-qi, et al. Degradation of Patulin in Fruit Juice by A Lactic Acid Bacteria Strain *Lactobacillus Casei* Yzu01[J]. *Food Control*, 2020, 112: 107147.
- [24] SARAVANAKUMAR K, SATHIYASEELAN A, MARIADOSS A V A, et al. *Lactobacillus Rhamnosus* GG and Biochemical Agents Enrich the Shelf Life of Fresh-Cut Bell Pepper (*Capsicum Annuum* L Var *Grossum* (L) Sendt)[J]. *Foods*, 2020, 9(9): 1252.
- [25] KAMAL R M, ALNAKIP M E, ABD EL AAL S F, et al. Bio-Controlling Capability of Probiotic Strain *Lactobacillus Rhamnosus* against Some Common Foodborne Pathogens in Yoghurt[J]. *International Dairy Journal*, 2018, 85: 1—7.
- [26] ARTUR MIKICIŃSKI P S. Perspectives on the Use of Bacteria in the Protection of Apple and Pear Against Fire Blight (*Erwinia Amylovora*)[J]. *Progress in Plant Protection*, 2018, 58(1): 68—75.
- [27] AIELLO D, RESTUCCIA C, STEFANI E, et al. Post-harvest Biocontrol Ability of *Pseudomonas Synxantha*

- against *Monilinia Fructicola* and *Monilinia Fructigena* on Stone Fruit[J]. *Postharvest Biology and Technology*, 2019, 149: 83—89.
- [28] PIETERSE C M, ZAMIOUDIS C, BERENDSEN R L, et al. Induced Systemic Resistance by Beneficial Microbes[J]. *Annu Rev Phytopathol*, 2014, 52: 347—375.
- [29] WANG Zhi-rong, MEI Xiao-fei, DU Mu-ying, et al. Potential Modes of Action of *Pseudomonas Fluorescens* Zx during Biocontrol of Blue Mold Decay on Postharvest Citrus[J]. *Journal of the Science of Food and Agriculture*, 2020, 100(2): 744—754.
- [30] WALLACE R L, HIRKALA D L, NELSON L M. Mechanisms of Action of Three Isolates of *Pseudomonas Fluorescens* Active against Postharvest Grey Mold Decay of Apple during Commercial Storage[J]. *Biological Control*, 2018, 117: 13—20.
- [31] WALLACE R L, HIRKALA D L, NELSON L M. Postharvest Biological Control of Blue Mold of Apple by *Pseudomonas Fluorescens* during Commercial Storage and Potential Modes of Action[J]. *Postharvest Biology and Technology*, 2017, 133: 1—11.
- [32] ALEGRE I, VINAS I, USALL J, et al. Antagonistic Effect of *Pseudomonas Graminis* Cpa-7 against Foodborne Pathogens in Fresh-Cut Apples under Simulated Commercial Conditions[J]. *Food Microbiol*, 2013, 33(2): 139—148.
- [33] ZHANG Yu, LI Teng-jie, LIU Yuan-fang, et al. Volatile Organic Compounds Produced by *Pseudomonas Chlororaphis* Subsp *Aureofaciens* Sps-41 as Biological Fumigants to Control *Ceratocystis Fimbriata* in Postharvest Sweet Potatoes[J]. *Journal of Agricultural and Food Chemistry*, 2019, 67(13): 3702—3710.
- [34] ZHANG Bo, LI Yao, ZHANG Yang-yan, et al. High-Cell-Density Culture Enhances the Antimicrobial and Freshness Effects of *Bacillus Subtilis* S1702 on Table Grapes (*Vitis Vinifera* Cv Kyoho)[J]. *Food Chemistry*, 2019, 286: 541—549.
- [35] MARI M, GUIZZARDI M, PRATELLA G C. Biological Control of Gray Mold in Pears by Antagonistic Bacteria[J]. *Biological Control*, 1996, 7(1): 30—37.
- [36] CHO G S, HANAK A, HUCH M, et al. Investigation into the Potential of Bacteriocinogenic *Lactobacillus Plantarum* BFE 5092 for Biopreservation of Raw Turkey Meat[J]. *Probiotics Antimicrob Proteins*, 2010, 2(4): 241—249.
- [37] LI Jun-sheng, BAI Jin, LI Shu-yi, et al. Effect of Lactic Acid Bacteria on the Postharvest Properties of Fresh Lotus Root[J]. *Postharvest Biology and Technology*, 2020, 160: 110983.
- [38] CHEN Cen, CAO Zheng, LI Jing, et al. A Novel Endophytic Strain of *Lactobacillus Plantarum* CM-3 with Antagonistic Activity against *Botrytis Cinerea* on Strawberry Fruit[J]. *Biological Control*, 2020, 148: 104306.
- [39] BARMAN S, GHOSH R, SENGUPTA S, et al. Long-term Storage of Post-Packaged Bread by Controlling Spoilage Pathogens Using *Lactobacillus Fermentum* C14 Isolated from Homemade Curd[J]. *PLOS One*, 2017, 12(8): e0184020.
- [40] LI Hong-juan, LIU Lu, ZHANG Shu-wen, et al. Potential Use of *Lactobacillus Casei* Ast18 as a Bioprotective Culture in Yogurt[J]. *Food Control*, 2013, 34(2): 675—680.
- [41] ABDEL-RAHMAN M A, SADEK Z I, AZAB M S, et al. Incorporation of Microencapsulated *Lactobacillus Rhamnosus* into Infant-Foods Inhibit Proliferation of Toxicogenic *Bacillus Cereus* Strains[J]. *Biocatalysis and Agricultural Biotechnology*, 2019, 18: 101013.
- [42] IGLESIAS M B, ABADIAS M, ANGUERA M, et al. Antagonistic Effect of Probiotic Bacteria against Foodborne Pathogens on Fresh-Cut Pear[J]. *LWT-Food Science and Technology*, 2017, 81: 243—249.
- [43] WANG Zhi-rong, JIANG Meng-yao, CHEN Ke-wei, et al. Biocontrol of *Penicillium Digitatum* on Postharvest Citrus Fruits by *Pseudomonas Fluorescens*[J]. *Journal of Food Quality*, 2018(2018): 2910481.
- [44] PRINCIPE A, FERNANDEZ M, TORASSO M, et al. Effectiveness of Tailocins Produced by *Pseudomonas Fluorescens* Sf4c in Controlling the Bacterial-Spot Disease in Tomatoes Caused by *Xanthomonas Vesicatoria*[J]. *Microbiological Research*, 2018, 212: 94—102.
- [45] IGLESIAS M B, LOPEZ M L, ECHEVERRIA G, et al. Evaluation of Biocontrol Capacity of *Pseudomonas Graminis* Cpa-7 against Foodborne Pathogens on Fresh-Cut Pear and Its Effect on Fruit Volatile Compounds[J]. *Food Microbiol*, 2018, 76: 226—236.
- [46] COLLAZO C, ABADIAS M, AGUILÓ-AGUAYO I, et al. Studies on the Biocontrol Mechanisms of *Pseudomonas Graminis* Strain CPA-7 Against Food-Borne Pathogens In Vitro and on Fresh-Cut Melon[J]. *LWT-Food Science and Technology*, 2017, 85: 301—308.
- [47] MIGUELEZ-SIERRA Y, ACEBO-GUERRERO Y, EL JAZIRI M, et al. *Pseudomonas Chlororaphis* Cp07 Strain Reduces Disease Severity Caused by *Phytophthora Palmivora* in Genotypes of *Theobroma Cacao*[J]. *European Journal of Plant Pathology*, 2019, 155(4): 1133—1143.
- [48] 申顺善, 张莹莹, 张维娜, 等. 绿针假单胞菌 HL5-4 对番茄灰霉菌的抑制活性及其定殖能力[J]. *园艺学报*, 2016, 43(6): 1195—1202.
- SHEN Shun-shan, ZHANG Ying-ying, ZHANG Wei-na, et al. Antifungal Activity of *Pseudomonas Choloeaphtis* HL5-4 Against Tomato Gray Mold and Its Colonization Ability[J]. *Acta Horticulturae Sinica*, 2016, 43(6): 1195—1202.
- [49] 申顺善, 赵玉华, 张利敬, 等. 绿针假单胞菌

- HG28-5对辣椒疫病的抑制活性及其根际定殖特性的研究[J]. 园艺学报, 2013, 40(8): 1574—1582.
- SHEN Shun-shan, ZHAO Yu-hua, ZHANG Li-jing, et al. Inhibitory Activity of *Pseudomonas Chlororaphis* HG28-5 Against *Phytophthora* Blight of Pepper and Its Root Colonization Characteristic[J]. *Acta Horticulturae Sinica*, 2013, 40(8): 1574—1582.
- [50] ZHANG Hong-yin, WANG Lei, DONG Ying, et al. Postharvest Biological Control of Gray Mold Decay of Strawberry with *Rhodotorula Glutinis*[J]. *Biological Control*, 2007, 40(2): 287—292.
- [51] CHEN Pei-hua, CHEN Rou-yun, CHOU Jui-yu. Screening and Evaluation of Yeast Antagonists for Biological Control of *Botrytis Cinerea* on Strawberry Fruits[J]. *Mycobiology*, 2018, 46(1): 33—46.
- [52] ZHAO Lu-ning, ZHOU Qiu-yang, YANG Hui-hui, et al. Inhibitory Effect of *Pichia Guilliermondii* Y35-1 Against Postharvest Anthracnose Infection in Loquat Fruit and Its Effect on Quality Preservation[J]. *Shipin Kexue/Food Science*, 2019, 40(4): 170—177.
- [53] LIU Ye, WANG Wei-hao, ZHOU Ya-han, et al. Isolation, Identification and in Vitro Screening of Chongqing Orangery Yeasts for the Biocontrol of *Penicillium Digitatum* on Citrus Fruit[J]. *Biological Control*, 2017, 110: 18—24.
- [54] PEREZ M F, PEREZ IBARRECHE J, ISAS A S, et al. Antagonistic Yeasts for the Biological Control of *Penicillium Digitatum* on Lemons Stored under Export Conditions[J]. *Biological Control*, 2017, 115: 135—140.
- [55] MEDINA-CÓRDOVA N, ROSALES-MENDOZA S, HERNÁNDEZ-MONTIEL L G, et al. The Potential Use of *Debaryomyces Hansenii* for the Biological Control of Pathogenic Fungi in Food[J]. *Biological Control*, 2018, 121: 216—222.
- [56] IACUMIN L, MANZANO M, ANDYANTO D, et al. Biocontrol of Ochratoxigenic Moulds (*Aspergillus Ochraceus* and *Penicillium Nordicum*) by *Debaryomyces Hansenii* and *Saccharomycopsis Fibuligera* during Speck Production[J]. *Food Microbiol*, 2017, 62: 188—195.
- [57] ALVAREZ M, NUNEZ F, DELGADO J, et al. Competitiveness of Three Biocontrol Candidates against Ochratoxigenic *Penicillium Nordicum* under Dry-Cured Meat Environmental and Nutritional Conditions[J]. *Fungal Biology*, 2021, 125(2): 134—142.
- [58] PEROMINGO B, ANDRADE M J, DELGADO J, et al. Biocontrol of Aflatoxigenic *Aspergillus Parasiticus* by Native *Debaryomyces Hansenii* in Dry-Cured Meat Products[J]. *Food Microbiol*, 2019, 82: 269—276.
- [59] SUI Yuan, SUN Zhi-qiang, ZOU Yu-ping, et al. The Rlm1 Transcription Factor in *Candida Oleophila* Contributes to Abiotic Stress Resistance and Biocontrol Efficacy against Postharvest Gray Mold of Kiwifruit[J]. *Postharvest Biology and Technology*, 2020, 166: 111222.
- [60] NIE Xiao-bao, ZHANG Chang-feng, JIANG Chang-xing, et al. Trehalose Increases the Oxidative Stress Tolerance and Biocontrol Efficacy of *Candida Oleophila* in the Microenvironment of Pear Wounds[J]. *Biological Control*, 2019, 132: 23—28.
- [61] AHIMA J, ZHANG Xiao-yun, YANG Qi-ya, et al. Biocontrol Activity of *Rhodotorula Mucilaginosa* Combined with Salicylic Acid against *Penicillium Digitatum* Infection in Oranges[J]. *Biological Control*, 2019, 135: 23—32.
- [62] WAFAR M H, A-E-K F, SINGER S M, et al. Production and Optimization of *Rhodotorula Glutinis* for Biocontrolling of Strawberry Blight[J]. *Research Journal of Pharmaceutical, Biological and Chemical Sciences*, 2014, 5(6): 599—620.
- [63] THANGAVELU R, GOPI M. Combined Application of *Nativetrichoderma* Isolates Possessing Multiple Functions for the Control of *Fusarium Wilt* Disease in Banana Cv Grand Naine[J]. *Biocontrol Science and Technology*, 2015, 25(10): 1147—1164.
- [64] EL-SAYED S F, EL-HELALY M A, EMAM M S, et al. Effect of Some Post-Cold Storage Treatments on Shelf Life of Sweet Potato Roots[J]. *Journal of Horticultural Science & Ornamental Plants*, 2013, 5(3): 160—170.
- [65] DANIA V O, FADINA O O, AYODELE M, et al. Evaluation of Isolates of *Trichoderma*, *Pseudomonas* and *Bacillus* Species as Treatment for the Control of Post-Harvest Fungal Rot Disease of Yam (*Dioscorea Spp*)[J]. *Archives of Phytopathology and Plant Protection*, 2016, 49(17): 456—470.
- [66] SANGEETHA G, USHARANI S, MUTHUKUMAR A. Biocontrol with *Trichoderma* Species for the Management of Postharvest Crown Rot of Banana[J]. *Phytopathologia Mediterranea*, 2009, 48(2): 214—225.
- [67] DANIA V O. Bioefficacy of *Trichoderma* Species against Important Fungal Pathogens Causing Post-Harvest Rot in Sweet Potato (*Ipomoea Batatas* (L) Lam)[J]. *Journal of the Bangladesh Agricultural University*, 2019, 17(4): 446—453.
- [68] EL-KATATNY M H, ALI B A, EMAM A S, et al. Biological Effects of Two *Trichoderma Harzianum* Isolates and Mint Oil on Some Postharvest Fungal Pathogens of Sugar Beet[J]. *Journal of Advanced Biomedical and Pharmaceutical Sciences*, 2020, 3(2): 65—79.
- [69] KAKVAN N, HEYDARI A, ZAMANIZADEH H R, et al. Development of New Bioformulations Using *Trichoderma* and *Talaromyces* Fungal Antagonists for Biological Control of Sugar Beet Damping-off Disease[J]. *Crop Protection*, 2013, 53: 80—84.
- [70] LU Zhi-xiang, TU Guang-ping, ZHANG Ting, et al. Screening of Antagonistic *Trichoderma* Strains and Their Application for Controlling Stalk Rot in Ma-

- ize[J]. *Journal of Integrative Agriculture*, 2020, 19(1): 145—152.
- [71] JAMBHULKAR P P, SHARMA P, MANOKARAN R, et al. Assessing Synergism of Combined Applications of *Trichoderma Harzianum* and *Pseudomonas Fluorescens* to Control Blast and Bacterial Leaf Blight of Rice[J]. *European Journal of Plant Pathology*, 2018, 152(3): 747—757.
- [72] ELSHERBINY E A, AMIN B H, ALEEM B, et al. *Trichoderma* Volatile Organic Compounds as A Biofumigation Tool Against Late Blight Pathogen *Phytophthora Infestans* in Postharvest Potato Tubers[J]. *Journal of Agricultural and Food Chemistry*, 2020, 68(31): 8163—8171.
- [73] ZHAO Yan, LI Yan-fei, YIN Jing-jing. Effects of Hot Air Treatment in Combination with *Pichia Guilliermondii* on Postharvest Preservation of Peach Fruit[J]. *Journal of the Science of Food and Agriculture*, 2019, 99(2): 647—655.
- [74] ZHANG Xue-mei, ZONG Yuan-yuan, LI Zhi-cheng, et al. Postharvest *Pichia Guilliermondii* Treatment Promotes Wound Healing of Apple Fruits[J]. *Postharvest Biology and Technology*, 2020, 167: 111228.
- [75] ABDEL-RAHIM I R, ABO-ELYOUSR K A M. Using of Endophytic *Saccharomycopsis Fibuligera* and Thyme Oil for Management of Gray Mold Rot of Guava Fruits[J]. *Biological Control*, 2017, 110: 124—131.
- [76] DE SOUZA DE AZEVEDO P O, CONVERTI A, GIERUS M, et al. Application of Nisin as Biopreservative of Pork Meat by Dipping and Spraying Methods[J]. *Brazilian Journal of Microbiology*, 2019, 50(2): 523—526.
- [77] CHANG S S, LU W Y, PARK S H, et al. Control of Foodborne Pathogens on Ready-to-Eat Roast Beef Slurry by E-Polylysine[J]. *International Journal of Food Microbiology*, 2010, 141(3): 236—241.
- [78] NIU Xiao-di, LI Li-Zhu, XI Juan, et al. An Antimicrobial Agent Prepared by N-Succinyl Chitosan Immobilized Lysozyme and Its Application in Strawberry Preservation[J]. *Food Control*, 2020, 108: 106829.
- [79] 陈智理, 李健, 郭静婕, 等. 乳酸链球菌素在食品中的应用研究[J]. *广西轻工业*, 2011(8): 6—7.
- CHEN Zhi-li, LI Jian, GUO Jing-jie, et al. Application of Nisin in Food[J]. *Guangxi Journal of Light Industry*, 2011(8): 6—7.
- [80] 蓝蔚青, 冯豪杰, 刘大勇, 等. 微生物源生物保鲜剂对水产品腐败菌作用机制研究进展[J]. *包装工程*, 2020, 41(5): 31—38.
- LAN Wei-qing, FENG Hao-jie, LIU Da-yong, et al. Research Progress on Mechanism of Microbial Source Bio-Preservatives on Spoilage Bacteria of Aquatic Products[J]. *Packaging Engineering*, 2020, 41(5): 31—38.
- [81] IBARRA-SANCHEZ L A, EL-HADDAD N, MAHMOUD D, et al. Invited Review: Advances in Nisin Use for Preservation of Dairy Products[J]. *Journal of Dairy Science*, 2020, 103(3): 2041—2052.
- [82] 高蕾蕾, 李迎秋. 乳酸链球菌素及其在食品中的应用研究进展[J]. *中国调味品*, 2017, 42(3): 157—165.
- GAO Lei-lei, LI Ying-qiu. Rarch Progress of Nisin and Its Application in Food Industry[J]. *China Condiment*, 2017, 42(3): 157—165.
- [83] FENG Y, IBARRA-SÁNCHEZ L A, LUU L, et al. Co-Assembly of Nisin and Zein in Microfluidics for Enhanced Antilisterial Activity in Queso Fresco[J]. *Lwt Food Science & Technology*, 2019, 111: 355—362.
- [84] HUQ T, RIEDL B, BOUCHARD J, et al. Microencapsulation of Nisin in Alginate-Cellulose Nanocrystal (Cnc) Microbeads for Prolonged Efficacy against *Listeria Monocytogenes*[J]. *Cellulose*, 2014, 21(6): 4309—4321.
- [85] PAN Dan, ZHANG Dong, HAO Li-min, et al. Protective Effects of Soybean Protein and Egg White Protein on the Antibacterial Activity of Nisin in the Presence of Trypsin[J]. *Food Chemistry*, 2018, 239: 196—200.
- [86] LIANG Zheng-rong, HSIAO H I, JHANG D J. Synergistic Antibacterial Effect of Nisin, Ethylenediaminetetraacetic Acid, and Sulfite on Native Microflora of Fresh White Shrimp during Ice Storage[J]. *Journal of Food Safety*, 2020, 40(2): e12794.
- [87] 杜国丰, 陈红漫, 阚国仕. ϵ -聚赖氨酸的应用研究进展[J]. *轻工科技*, 2015(11): 25—26.
- DU Guo-feng, CHEN Hong-man, KAN Guo-shi. Research Progress on Application of ϵ -Polylysine[J]. *Light Industry Science and Technology*, 2015(11): 25—26.
- [88] 齐子琦, 秦子晋, 黄永震, 等. 生物防腐剂 ϵ -聚赖氨酸的研究进展[J]. *食品工业*, 2019, 40(10): 289—294.
- QI Zi-qi, QIN Zi-jin, HUANG Yong-zhen, et al. Research Progress of Biological Preservative ϵ -Polylysine[J]. *Food Industry*, 2019, 40(10): 289—294.
- [89] 刘琴英, 翁建超, 谢斯超, 等. ϵ -聚赖氨酸研究进展[J]. *科技视界*, 2012(23): 398—399.
- LIU Qin-ying, WENG Jian-chao, XIE Si-chao, et al. Research Progress of ϵ -Poly-L-Lysine[J]. *Science & Technology Vision*, 2012(23): 398—399.
- [90] LIN Lin, GU Yu-lei, LI Chang-zhu, et al. Antibacterial Mechanism of ϵ -Poly-Lysine Against *Listeria Monocytogenes* and Its Application on Cheese[J]. *Food Control*, 2018, 91: 76—84.
- [91] HUANG Ming-yuan, WANG Hu-hu, XU Xing-lian, et al. Effects of Nanoemulsion-Based Edible Coatings with Composite Mixture of Rosemary Extract and ϵ -Poly-Lysine on the Shelf Life of Ready-to-Eat Carbonado Chicken[J]. *Food Hydrocolloids*, 2020, 102: 105576.

- [92] 杨云斌, 周桂飞, 周斌. ϵ -聚赖氨酸对米饭保鲜效果的研究[J]. 食品工业科技, 2010, 31(9): 77—79.
YANG Yun-bin, ZHOU Gui-fei, ZHOU Bin. Study on Preservation of Cooked-Rice with ϵ -Poly-Lysine[J]. Science and Technology of Food Industry, 2010, 31(9): 77—79.
- [93] LI Ying-qiu, FENG Jian-ling, HAN Qing, et al. Effects of ϵ -Poly-Lysine on Physicochemical Characteristics of Chilled Pork[J]. Food Bioprocess Technol, 2014, 7: 2507—2515.
- [94] GUO Liang, MENG Yue-cheng, FANG Sheng. Physicochemical and Antimicrobial Properties of Epsilon-Polylysine/Carboxymethyl Chitosan Polyelectrolyte Complexes and Their Effect Against Spoilage Microorganisms in Raw Pork[J]. Food & Function, 2017, 8(6): 2243—2248.
- [95] SHAO Zhi-peng, YANG Yi, FANG Sheng, et al. Mechanism of the Antimicrobial Activity of Whey Protein-Epsilon-Polylysine Complexes Against Escherichia Coli and Its Application in Sauced Duck Products[J]. International Journal of Food Microbiology, 2020, 328: 108663.
- [96] LIU Jian-hua, XIAO Jie, LI Feng, et al. Chitosan-sodium Alginate Nanoparticle as a Delivery System for ϵ -Poly-Lysine: Preparation, Characterization and Antimicrobial Activity[J]. Food Control, 2018, 91: 302—310.
- [97] 孙浩, 石玉刚, 朱陈敏, 等. 溶菌酶的修饰、功能特性及其在食品保鲜中的应用研究进展[J]. 食品科学, 2019, 40(21): 334—342.
SUN Hao, SHI Yu-gang, ZHU Chen-min, et al. A Review of the Modification, Functional Characteristics and Application in Food Preservation of Lysozyme[J]. Food Science, 2019, 40(21): 334—342.
- [98] PELLEGRINI A T U, FELLEBERG R V, WILD P. Bactericidal Activities of Lysozyme and Aprotinin Against Gram-Negative and Gram-Positive Bacteria Related to Their Basic Character[J]. The Journal of Applied Bacteriology, 1992, 72(3): 180—187.
- [99] LIU Jun-hong, WANG Kun-yu. Technology Optimization of Lysozyme's Fresh Maintaining Effect on Apple[J]. Journal of Food Science, 2016, 81(11): 2872—2875.
- [100] XU Fang-xu, LIU Shi-yang, LIU Ye-fei, et al. Effectiveness of Lysozyme Coatings and 1-MCP Treatments on Storage and Preservation of Kiwifruit[J]. Food Chemistry, 2019, 288: 201—207.
- [101] WANG Zhe, HU Shuai-feng, GAO Yu-peng, et al. Effect of Collagen-Lysozyme Coating on Fresh-Salmon Fillets Preservation[J]. LWT-Food Science and Technology, 2017, 75: 59—64.
- [102] IBRAHIM H R, KATO A, KOBAYASHI K. Antimicrobial Effects of Lysozyme Against Gram-Negative Bacteria due to Covalent Binding of Palmitic Acid[J]. Journal of Agricultural and Food Chemistry, 1991, 39(11): 2077—2082.
- [103] JIANG Sui-sui, QIN Yang, YANG Jie, et al. Enhanced Antibacterial Activity of Lysozyme Immobilized on Chitin Nanowhiskers[J]. Food Chemistry, 2017, 221: 1507—1513.
- [104] HASHEMI M, AMINLARI M, FOROUZAN M, et al. Production and Application of Lysozyme-Gum Arabic Conjugate in Mayonnaise as a Natural Preservative and Emulsifier[J]. Polish Journal of Food and Nutrition Sciences, 2018, 68(1): 33—43.
- [105] ERNEST V, GAJALAKSHMI S, MUKHERJEE A, et al. Enhanced Activity of Lysozyme-Agnp Conjugate with Synergic Antibacterial Effect without Damaging the Catalytic Site of Lysozyme[J]. Artif Cells Nanomed Biotechnol, 2014, 42(5): 336—343.
- [106] 庞莉, 陆建安, 王森, 等. 阿魏酸化学修饰溶菌酶及扩展抑菌谱的研究[J]. 食品工业科技, 2019, 30(11): 184—187.
PANG Li, LU Jian-an, WANG Miao, et al. Study on Chemical Modification of Lysozyme with Ferulic Acid and Exhibiting an Enhanced Antimicrobial Action[J]. Science and Technology of Food Industry, 2019, 30(11): 184—187.
- [107] 宋雪健, 张东杰, 王洪江, 等. 天然生物抗菌剂纳他霉素在食品中的应用及研究进展[J]. 保鲜与加工, 2017, 17(5): 129—135.
SONG Xue-jian, ZHANG Dong-jie, WANG Hong-jiang, et al. Application and Research Progress of Natural Bio-antibacterial Agent Natamycin in Food[J]. Storage and Process, 2017, 17(5): 129—135.
- [108] FENTE-SAMPAYO C A, VAZQUEZ-BELDA B, FRANCO-ABUIN C, et al. Distribution of Fungal Genera in Cheese and Dairies. Sensitivity to Potassium Sorbate and Natamycin[J]. Archiv Für Lebensmittelhygiene, 1995, 46(3): 49—72.
- [109] 李东, 杜连祥, 路福平, 等. 纳他霉素的抑菌谱及最小抑菌浓度[J]. 食品工业科技, 2004, 25(7): 143—144.
LI Dong, DU Lian-xiang, LU Fu-ping, et al. Antimicrobial Spectrum and Minimum Inhibitory Concentration of Natamycin[J]. Science and Technology of Food Industry, 2004, 25(7): 143—144.
- [110] GARAVAGLIA J, PINTO L M N, SOUZA D D, et al. Natamycin and Nisin to Improve Shelf Life and Minimize Benzene Generation in Lemon Soft Drinks[J]. Food Science and Technology, 2019, 39(2): 274—279.
- [111] OLLÉ RESA C P, JAGUS R J, GERSCHENSON L N. Natamycin Efficiency for Controlling Yeast Growth in Models Systems and on Cheese Surfaces[J]. Food Control, 2014, 35(1): 101—108.
- [112] DUAN Dan-ping, QIAO Yong-jin, LU Li-sha, et al. Fresh-Keeping Effect of Natamycin and Chitosan Compound on Strawberry during Storage[J]. Journal

- of Hunan Agricultural University, 2010, 36(2): 237—241.
- [113] MOSTAFA N, KIRRELLA G, AIDEIA H, et al. Assessment of Mould Contamination of Tilapia Nilotica and Mugil Cephalus Fish and Trials to Reduce Using Natamycin[J]. Slovenian Veterinary Research, 2019, 56(22): 515—522.
- [114] HASTAOGLU E, GÖKSEL SARAÇ M, SABA KESKİN Z, et al. The Effects of Nisin and Natamycin on the Microbiological, Chemical and Sensorial Qualities of Meatballs[J]. Cumhuriyet Science Journal, 2017, 38(4): 845—855.
- [115] 姜爱丽, 胡文忠, 田密霞, 等. 纳他霉素在草莓保鲜中应用的研究[J]. 食品科学, 2007, 28(12): 515—520. JIANG Ai-li, HU Wen-zhong, TIAN Mi-xia, et al. Study on Utilization of Natamycin in Storage of Strawberry Fruit[J]. Food Science, 2007, 28(12): 515—520.
- [116] WEN Ming, LIN Xian, YU Yuan-shan, et al. Natamycin Treatment Reduces the Quality Changes of Post-harvest Mulberry Fruit during Storage[J]. Journal of Food Biochemistry, 2019, 43(8): 12934.
- [117] NYMAN P J, WAMER W G, BEGLEY T H, et al. Evaluation of Accelerated Uv and Thermal Testing for Benzene Formation in Beverages Containing Benzoate and Ascorbic Acid[J]. Journal of Food Science, 2010, 75(3): 263—267.
- [118] ROMERO V, BORNEO R, PASSALACQUA N, et al. Biodegradable Films Obtained from Triticale (X Triticosecale Wittmack) Flour Activated with Natamycin for Cheese Packaging[J]. Food Packaging and Shelf Life, 2016, 10: 54—59.
- [119] HAO Xiao-lei, ZHANG Jiao-jiao, LI Xi-hong, et al. Application of A Chitosan Coating as A Carrier for Natamycin to Maintain the Storage Quality of Ground Cherry (Physalis Pubescens L)[J]. Journal of Zhejiang University-SCIENCE B, 2017, 18(9): 807—815.
- [120] FUCIÑOS C, MÍGUEZ M, CERQUEIRA M A, et al. Functional Characterisation and Antimicrobial Efficiency Assessment of Smart Nanohydrogels Containing Natamycin Incorporated into Polysaccharide-Based Films[J]. Food and Bioprocess Technology, 2015, 8(7): 1430—1441.
- [121] 周倩倩, 谢晶. 含有壳聚糖的保鲜剂在水产品保鲜中应用的研究进展[J]. 食品工业科技, 2019, 40(1): 347—351. ZHOU Qian-qian, XIE Jing. Research Progress of Preservatives Containing Chitosan in Fresh-Keeping of Aquatic Products[J]. Science and Technology of Food Industry, 2019, 40(1): 347—351.
- [122] LOPES N A, BARRETO PINILLA C M, BRANDELLI A. Antimicrobial Activity of Lysozyme-Nisin Co-Encapsulated in Liposomes Coated with Polysaccharides[J]. Food Hydrocolloids, 2019, 93: 1—9.
- [123] YU H H, KIM Y J, PARK Y J, et al. Application of Mixed Natural Preservatives to Improve the Quality of Vacuum Skin Packaged Beef during Refrigerated Storage[J]. Meat Science, 2020, 169: 108219.
- [124] 张群利, 崔琳琳, 李春伟, 等. 壳聚糖复合生物保鲜剂对池沼公鱼保鲜品质的影响[J]. 中国水产, 2012(10): 68—69. ZHANG Qun-li, CUI Lin-lin, LI Chun-wei, et al. The Effect of Chitosan Compound Biological Preservative on the Fresh Keeping Quality of Pond Marsh Male Fish[J]. China Fisheries, 2012(10): 68—69.
- [125] 徐畅, 蔡明迪, 刘秋, 等. 菊粉天然复合保鲜剂对冷鲜猪肉保鲜效果的影响[J]. 包装工程, 2020, 41(19): 8—16. XU Chang, CAI Ming-di, LIU Qiu, et al. Effect of Inulin Nature Compound Preservative on Chilled Pork during Refrigerated Storage[J]. Packaging Engineering, 2020, 41(19): 8—16.
- [126] 曹森, 李江阔, 张鹏, 等. 不同保鲜剂对贵长猕猴桃后熟品质的影响[J]. 包装工程, 2019, 40(19): 50—56. CAO Sen, LI Jiang-kuo, ZHANG Peng, et al. Effects of Different Preservatives on Post-Ripening Quality of "Gui Chang" Kiwifruit[J]. Packaging Engineering, 2019, 40(19): 50—56.