·综 述·

酶制剂清除食源性致病菌生物被膜的研究进展

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摘 要: 食源性致病菌对人类健康与公众安全造成了极大的危害，形成生物被膜加剧了它们的致病与耐药风险。酶具有高度专一性，可靶向作用于生物被膜中的特殊物质，从而清除食源性致病菌的生物被膜，具有重要的科研价值和广泛的应用前景。因此，文中系统地综述了相关酶制剂清除食源性致病菌生物被膜的研究进展。根据酶制剂的不同作用靶点，着重介绍了群体感应抑制酶、环二鸟苷酸代谢酶、胞外基质水解酶等酶制剂的研究现状。文中还针对抗生物被膜酶制剂的未来研究方向进行了展望，旨在为食源性致病菌生物被膜的有效控制提供新的技术与策略。

关键词: 酶制剂，食源性致病菌，生物被膜，靶向清除

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Enzyme-based targeted disintegration of biofilms formed by food-borne pathogens: a review

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Abstract: Food-borne pathogens pose great risks to human health and public safety, and the formation of biofilm exacerbates their pathogenicity and antimicrobial resistance. Enzymes can target special substances in the biofilm to disintegrate the biofilm of food-borne pathogens, which has great potential for applications. This review summarized the progress of using enzymes to disintegrate the biofilms of food-borne pathogens, highlighting quorum-quenching enzymes, C-di-GMP metabolic enzymes, as well as extracellular matrix hydrolases. Finally, challenges and perspectives on developing enzymes into effective products for disintegrating the biofilms of food-borne pathogens were discussed.

Keywords: enzymes, foodborne pathogens, biofilm, targeted disintegration

食源性致病菌导致的食源性疾病是全世界共同关注的焦点、难点问题。据世界卫生组织(World Health Organization，WHO) 统计，全球每年约有 42 万人因感染食源性致病菌而死亡[1]。食源性致病菌能够在食品基质及相关加工器械表面形成生物被膜，这种特殊的结构可以保护病原体免受抗生素、洗涤剂、高温高压等不利外界条件的侵害[2]。生物被膜是由细菌细胞黏附于接触物体表面，通过分泌多糖、蛋白、脂质等胞外聚合物而形成的多细胞聚集体[3-4]。研究表明，当细菌存在于生物被膜中时，它们对抗生素的耐药性会提高 10–1 000 倍[5-6]，笔者课题组研究表明，生物被膜可为细菌提供耐药基因突变和水平基因转移的机会，加剧食源性致病菌的耐药风险[7-8]。此外，生物被膜的形成还容易引起食品腐败、食品相关设备腐蚀、交叉污染等严重的食品安全问题，造成巨大的经济损失[9]。

传统食品工业中，常用化学试剂或物理杀菌等方式来减轻生物被膜的危害[10]，但是残留的化学试剂容易导致环境污染[11]，而一些物理杀菌方式有可能破坏食品的质构、改变食品的风味[12]。酶与底物的结合具有高度特异性，能够靶向作用于生物被膜的胞外基质，降解生物被膜中的群体感应信号分子，阻断细胞与细胞之间的交流，从而加速生物被膜的瓦解[13]。酶制剂具有专一、高效、无污染等优势，已经广泛应用于食品工业之中[14]。图 1 总结了酶制剂清除食源性致病菌生物被膜的作用机制，首先酶通过渗透作用进入病原体的生物被膜，然后特异性地识别特殊物质，从根本上清除食源性致病菌的生物被膜。

因此，本文针对食源性致病菌生物被膜的危害，结合笔者课题组的相关研究，综述了应用酶制剂清除食源性致病菌生物被膜的研究进展，并根据酶制剂作用靶点及机制的不同，系统地将其分类为群体感应抑制酶、环二鸟苷酸代谢酶、胞外基质水解酶等，详细地阐述了这些酶的来源、分类、作用靶点及应用，并对其未来研究方向进行了展望。本文旨在为食源性致病菌生物被膜的有效控制提供新的思路，为抗生物被膜酶制剂的进一步开发与应用奠定理论基础。

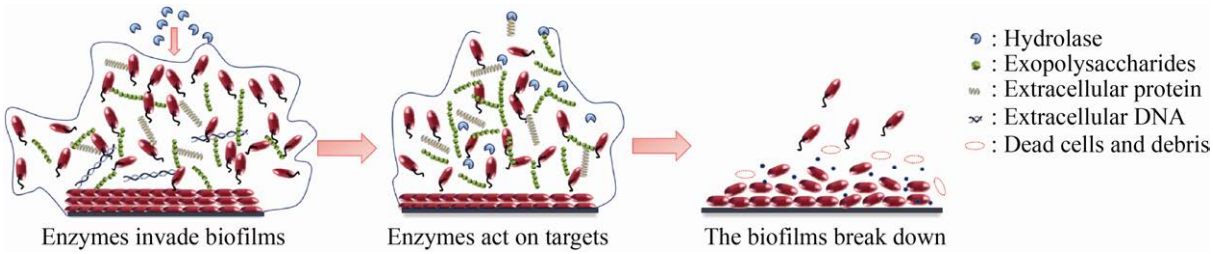

图 1　酶制剂清除食源性致病菌生物被膜的动态过程

Fig. 1　Process of enzyme-targeted disintegration of biofilm of food-borne pathogens.

1　食源性致病菌生物被膜的危害

生物被膜能够导致食源性致病菌产生耐药性、增强致病菌对宿主细胞的侵袭力、造成食品工业中交叉污染等。首先，生物被膜的特殊微观结构和代谢状态，是细菌耐药性形成的因素之一[15]。Hall 等[6]发现假单胞菌携带胞外多糖合成基因座(*psl*, polysaccharide synthesis locus)，能够合成 Psl 胞外多糖，在该菌生物被膜形成阶段对多粘菌素 B、环丙沙星等产生耐药性[13]。笔者课题组研究表明，副溶血性弧菌 *Vibrio parahaemolyticus* 和单核细胞增生李斯特菌 *Listeria monocytogenes* 形成混合生物被膜时，耐药性发生显著变化[8]。其次，生物被膜能增加致病菌毒性，提升其对宿主细胞的侵袭力[16]。例如，生物被膜会促使金黄色葡萄球菌 *Staphylococcus aureus* 释放大量肠毒素，导致食物中毒[17]。被膜态的霍乱弧菌 *Vibrio cholerae* 感染细胞时，毒力基因表达量显著提升[18]。第三，食源性致病菌可在食品加工各个环节形成生物被膜，导致交叉污染[9]。例如，金黄色葡萄球菌、假单胞菌 *Pseudomonas* 等会粘附于乳品生产线、运输管道等表面形成生物被膜，导致乳品的交叉污染和食品安全问题[19]。笔者课题组研究表明，副溶血性弧菌易在食品加工材料[20-21]及水产品[22]表面形成生物被膜，并能与霍乱弧菌[23]、单核细胞增生李斯特菌[8]等形成混合生物被膜，引起食品工业中的交叉污染问题。

2　用于清除生物被膜的酶制剂

针对以上食源性致病菌生物被膜的严重危害，研究人员开发了不同种类的抗生物被膜酶制剂，为生物被膜的有效瓦解提供了新的技术和策略[14]。本文进一步对基于不同作用靶点的抗生物被膜酶制剂进行总结，其具体的作用靶点如图 2 所示，这些酶制剂通过抑制或水解致病菌生物被膜形成过程中的必需物质，达到清除生物被膜的目的。其中包括群体感应抑制酶、环二鸟苷酸代谢酶和胞外基质水解酶等。

2.1　群体感应抑制酶

群体感应 (Quorum sensing，QS) 是细菌之间相互交流的一种方式，大部分微生物可分泌信号分子调控细菌的行为，如形成生物被膜、产生毒素、产生抗菌肽等。群体感应信号分子可分为 3 类：一是 N-乙酰高丝氨酸内酯 (N-acyl-homoserine lactones，AHLs)；二是自诱导肽 (Autoinducing peptide，AIP)；三是自诱导物-2 (Autoinducer-2，AI-2)[24]。AHLs 是一类在革兰氏阴性菌群体感应系统中最典型的信号分子，在细菌生物被膜的形成中起着关键作用[25]。因此，靶向作用于群体感应信号分子的酶制剂能有效地清除食源性致病菌生物被膜。

群体感应抑制酶 (Quorum quenching enzyme，QQ 酶) 的作用机制是通过降解群体感应信号分子，达到抑制生物被膜形成的目的[26]。表 1 总结

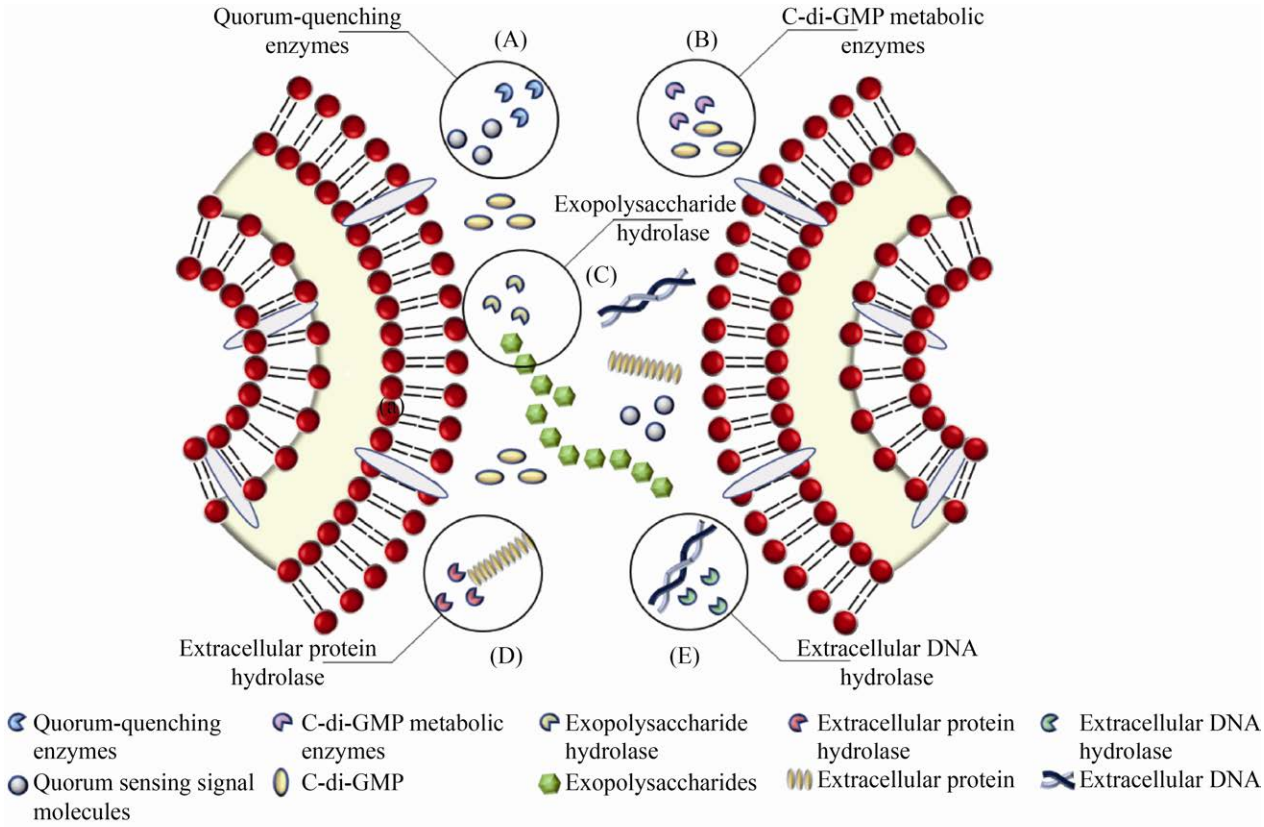

图 2　不同靶点清除食源性致病菌生物被膜的酶制剂

Fig. 2　Categories of enzymes that are able to targeted-disintegrate the biofilm of food-borne pathogens. (A) Enzymes act at quorum sensing signaling molecules. (B) Enzymes act at C-di-GMP. (C) Hydrolases act at exopolysaccharides. (D) Hydrolases act at extracellular proteins. (E) Hydrolases act at extracellular DNA.

了 QQ 酶的分离来源、致病菌种类及靶向作用位点。目前，大多数 QQ 酶的研究都以 AHLs 为作用位点，可以从芽孢杆菌、假单胞菌、拟杆菌等多种来源获得。根据酶制剂的特性，可系统地将降解 AHLs 的酶分为 3 类：AHLs-内酯酶、AHLs-酰化酶和 AHLs-氧化还原酶。

2.1.1　AHLs-内脂酶

AiiA 酶是第一个被分离获得的 AHLs-内脂酶，来源于芽孢杆菌 240B1，通过水解方式裂解 AHLs 中的高丝氨酸内酯环，降低 QS 分子的有效性，对胡萝卜软腐欧文氏菌 *Erwinia carotovora* 的生物被膜有明显的抑制效果[27]。Chen 等[28]从芽孢杆菌 B546 中也分离获得了 AiiA 酶，将其命名为 AiiAB546，可有效抑制嗜水气单胞菌 *Aeromonas hydrophila* 的生物被膜。Wang 等[29]在砖红色微杆菌 StLB037 *Microbacterium testaceum* 的染色体上发现了一段编码 AHLs-内脂酶的基因 (*aiiM*)，通过重组表达获得了 AiiM 酶，可用于胡萝卜软腐果胶杆菌 *Pectobacterium carotovorum* 生物被膜的控制。

目前，在根瘤菌 *Rhizobium* spp.、硝化杆菌 *Bacterium nitrobacter*、荧光假单胞菌 *Pseudomonas fluorescens* 等细菌中也发现了 AHLs-内脂酶。例如，根瘤菌 NGR234 产生的 AHLs-内脂酶 DlhR 和 QsdR1，可通过抑制群体感应来控制铜绿假单胞菌 *Pseudomonas fluorescens*、紫色色杆菌 *Chromobacterium violaceum* 和根癌农杆菌 *Agrobacterium tumefaciens* 的生物被膜[31]。研究人员分别从硝化杆菌 Nb-311A[32]和荧光假单胞菌[32]中分离获得了 BpiB01 和 BpiB04，能够降解 AHLs 分子并抑制铜绿假单胞菌生物被膜的形成。

表 1 群体感应抑制酶的种类、来源及作用靶点

Table 1 The sources, target bacteria, and target quorum-sensing (QS) molecules of quorum-quenching (QQ) enzymes

Quorum-quenching enzymes	Source	Target pathogenic bacteria	References
AHLs-lactonases			
AiiA	*Bacillus* spp. strain 240B1	*Erwinia carotovora*	[27]
$AiiA_{B546}$	*Bacillus* spp. strain B546	*Aeromonas hydrophila*	[28]
AiiM	*Microbacterium testaceum* strain StLB037	*Pectobacterium carotovorum*	[29]
AidH	*Ochrobactrum* spp. strain T63	*Pseudomonas fluorescens*	[30]
DlhR and QsdR1	*Rhizobium* spp. strain NGR234	*Pseudomona aeruginosa* *Chromobacterium violaceum* *Agrobacterium tumefaciens*	[31]
BpiB01	*Nitrobacter* spp. strain Nb-311A	*Pseudomona aeruginosa*	[32]
BpiB04	*Pseudomonas fluorescens*	*Pseudomona aeruginosa*	[32]
AHLs-acylase			
AiiD	*Variovorax paradoxus* VAI-C	*Pseudomona aeruginosa*	[33]
Aac	*Shewanella* spp. strain MIB015	*Vibrio anguillarum*	[34]
Aac	*Ralstonia solanacearum* strain GMI1000	*Chromobacterium violaceum*	[35]
HacB (PA0305)	*Pseudomonas aeruginosa* strain PAO1	*Pseudomonas aeruginosa*	[36]
PvdQ (PA2385)	*Pseudomonas aeruginosa* strain PAO1	*Pseudomonas aeruginosa*	[37]
Oxidoreductases			
BpiB09 (Short-chain dehydrogenase/reductase [SDR])	*Acidobacterium* spp. strain MP5ACTX8	*Pseudomonas aeruginosa*	[38]
Reductase	*Burkholderia* sp. GG4; *Klebsiella* sp. strain Se14; *Acinetobacter* spp. strain GG2	*Pseudomonas aeruginosa*; *Erwinia carotovora*	[39]

2.1.2 AHLs-酰化酶

AHLs-酰化酶属于 NTN-水解酶超家族，最早发现于争论贪噬菌 VAI-C *Variovorax paradoxus* 的生物被膜中，能水解 AHLs 酰基链和高丝氨酸部分之间的酰胺键[33]。Wahjud 等[36]从铜绿假单胞菌中获得的 HacB 酶，对铜绿假单胞菌的长链 AHLs 分解具有催化活性，能抑制该菌生物被膜的形成。Sio 等[37]从铜绿假单胞菌 PAO1 中获得的 PvdQ 酶，能降解该菌中含有 11–14 个碳链的 AHLs，降低细胞毒性。

2.1.3 AHLs-氧化还原酶

AHLs-氧化还原酶能够催化 AHLs 酰基侧链的氧化或还原[40-41]。目前，已在伯克霍尔德氏菌属 *Burkholderia* spp.、克雷伯氏菌属 *Klebsiella* spp.、不动杆菌属 *Acinetobacter* spp.等细菌中发现 AHLs-氧化还原酶。例如，Bijtenhoorn 等[38]从酸杆菌属 MP5ACTX8 *Acidobacterium* spp.中分离获得的 BpiB09 短链脱氢酶，该酶在铜绿假单胞菌 PAO1 中的表达，导致了铜绿假单胞菌运动能力降低、生物被膜形成量减少。

2.2 基于环二鸟苷酸的生物被膜代谢酶

环二鸟苷酸 (C-di-GMP) 是著名的细菌第二信使，存在于所有细菌之中[42]，具有多种调节功能，如胞外聚合物 (EPS) 生物合成、生物被膜的形成、参与细胞的运动等。研究表明 C-di-GMP 水平升高可使生物被膜形成能力显著增强[43]，参与调节 C-di-GMP 浓度的代谢酶主要分为两类：二鸟苷酸环化酶 (Diguanylate cyclases，DGCs) 和磷酸二酯酶 (Phosphodiesterases，PDEs)。表 2 总结了具有代表性的 C-di-GMP 代谢酶，合理利

表 2 环二鸟苷酸代谢酶

Table 2 C-di-GMP metabolic enzymes

The type of enzyme	Name	Source	Function	Domain	References
Diguanylate cyclase	PleD	*Caulobacter crescentus*	C-di-GMP synthesis	GGDEF domain	[44]
	WspR	*Pseudomonas aeruginosa*	C-di-GMP synthesis; Biofilm formation and persistence	GGDEF domain	[45]
	DgcA, DgcB and DgcC	*Listeria monocytogenes*	Biofilm formation	GGDEF domain	[46]
Phosphodiesterase	YkuI	*Bacillus subtilis*	C-di-GMP hydrolysis	EAL domain	[47]
	BlrP1	*Klebsiella pneumoniae*	C-di-GMP hydrolysis was regulated by light	EAL domain	[48]
	Bd1817	Vermiculite isolated bacteria	Unknown	HD-GYP domain	[49]
	PdeB, PdeC and PdeD	*Listeria monocytogenes*	C-di-GMP hydrolysis	EAL domain	[46]

用 DGCs 和 PDEs 可加速 C-di-GMP 的降解，从而清除食源性致病菌的生物被膜。

在大多数细菌中，DGCs 参与 C-di-GMP 的催化合成，而 PDEs 则负责 C-di-GMP 的催化降解。DGCs 带有一个保守 GGDEF (Gly-Gly-Asp-Glu-Phe) 结构域[42]，能够催化两个 GTP 分子合成 C-di-GMP。PDEs 具有保守 EAL (Glu-Ala-Leu) 或 HD-GYP (His-Asp-Gly-Tyr-Pro) 结构域，可将 C-di-GMP 水解为线性的二聚鸟苷酸 (pGpG)，最后被寡核糖核酸酶分解为两个 GMP 分子。目前，尚未有研究将以上两种 C-di-GMP 代谢酶应用于致病菌生物被膜的控制，未来可根据这两种酶的生物学特性，催化加速 C-di-GMP 的合成与降解，达到清除生物被膜的目的。

2.3 基于胞外基质的生物被膜水解酶

胞外基质是生物被膜的主要成分，能为生物被膜中的致病菌提供保护，加强其对宿主免疫系统的防御和对抗生素的抵抗作用[50-51]。笔者课题组研究表明，胞外聚合物在副溶血性弧菌生物被膜形成过程中起决定性作用[52-53]。胞外基质水解酶的作用机制是利用酶的专一性，靶向作用于胞外基质成分，从而达到清除食源性致病菌生物被膜的目的。表 3 中总结了各种胞外基质水解酶的来源与作用靶点。胞外基质水解酶主要分为 3 类：

表 3 胞外基质水解酶的种类、来源和作用靶点

Table 3 The categories, sources and targets of extracellular matrix hydrolases

Extracellular matrix	Category	Source	Target/Matrix	References
Exopolysaccharides	Alginate lyase	*Pseudomonas aeruginosa*	β-glycoside bond that links a brown alginate polymer	[54]
	PslGh	*Pseudomonas aeruginosa*	Psl exopolysaccharides	[55]
	PelAh	*Pseudomonas aeruginosa*	Pel exopolysaccharides	[55]
	Lysozyme	Microbial, animal and plant extracts	Peptidoglycan layer of bacterial cell wall	[56]
	α-Amylase	*Bacillus subtilis*	α-1,4 glycosidic linkage	[57]
	Dispersing B	*Periodontal actinomycetes*	β-1,6-N-acetyl-D-glucosamine	[58]
Extracellular protein	*Bacillus subtilis* protease	*Bacillus*	Peptide bonds in protein structures	[59]
	Lysostaphin	*Staphylococcus aureus*	Pentapeptide bond of peptidoglycan layer	[60]
	Bacteriophage lysin	Bacteriophage Vb-SepiS-phiIPLA7	Bacterial cell walls based on peptidoglycan	[61]
Extracellular DNA	DNase	Widespread in living organisms	The phosphodiester linkage of DNA	[62]

胞外多糖水解酶、胞外蛋白水解酶和胞外 DNA 水解酶。

2.3.1 基于胞外多糖的生物被膜水解酶

胞外多糖是构筑食源性致病菌生物被膜的核心骨架，能帮助生物被膜中的细菌进行粘附，促进其在食品器械表面的定植。利用胞外多糖水解酶针对性地水解胞外多糖，有助于破坏细菌的附着，减少生物被膜的形成量。在铜绿假单胞菌的生物被膜基质中存在 3 种活性胞外多糖：褐藻胶、Psl 和 Pel (一种由 N-乙酰-D-氨基葡萄糖和 N-乙酰基-D-半乳糖胺组成的阳离子胞外多糖)[63]。研究表明，褐藻胶裂解酶 (AlsL) 破坏生物被膜中褐藻胶聚合物的作用位点是 β-糖苷键，可加速假单胞菌生物被膜的催化降解，从非生物表面剥离生物被膜[64-65]，并具有增强人体免疫细胞杀菌效果的功能[66]。Baker 等[55]研究发现，Pel 和 Psl 生物合成操纵子中分别存在一段可编码糖苷水解酶 PelAh 和 PslGh 的基因序列，这两种水解酶能够分别靶向降解生物被膜中的胞外多糖 Pel 和 Psl，抑制生物被膜的形成。此外，Asker 等[67]将糖苷水解酶 PslGh 固定在玻璃、PDMS 等材料表面，构建了一种铜绿假单胞菌生物被膜完全不能生长的新型生物材料。

2.3.2 基于胞外蛋白的生物被膜水解酶

胞外蛋白作为生物被膜胞外基质的主要成分之一，在食源性致病菌生物被膜的结构和功能中发挥重要作用[68]。蛋白水解酶能够通过水解氨基酸残基之间的特定肽键降解胞外蛋白，从而达到清除生物被膜的效果。枯草芽孢杆菌 *Bacillus subtilis* 蛋白酶[58]是一种能够攻击肽键的非特异性丝氨酸蛋白酶，能破坏假交替单胞菌 *Pseudoalteromonas* 的粘附性胞外蛋白，使生物被膜的结构分离。此外，溶葡萄球菌酶 (Lst)[69]是一种应用广泛的锌依赖性金属蛋白酶，可水解食源性致病菌肽聚糖层的五聚肽键，达到降解胞外蛋白的目的。

2.3.3 基于胞外 DNA 的生物被膜水解酶

胞外 DNA (eDNA) 是食源性致病菌生物被膜形成的关键元素[70]，在单核细胞增生李斯特菌[71]、铜绿假单胞菌[72]和金黄色葡萄球菌[73]生物被膜的粘附、构建和维护中起着重要作用，因此降解 eDNA 也是清除生物被膜的一种有效的方法。商用的脱氧核糖核酸酶 (DNase Ⅰ) 通常用于破坏 DNA 的磷酸二酯键，在 DNase Ⅰ处理革兰氏阴性菌和革兰氏阳性菌的实验中发现[74]，5 μg/mL 的 DNase Ⅰ可以使细菌的生物被膜量减少约 40%。此外，DNase Ⅰ还可与其他酶联合使用，Karygianni 等[75]研究发现 DNase Ⅰ和蛋白酶联合处理能破坏口腔生物被膜组成及结构的完整性。

2.4 其他类型的生物被膜水解酶

2.4.1 氧化还原酶

氧化还原酶是利用 H_2O_2 抑制细菌的生长繁殖，从而减少生物被膜的形成。葡萄糖氧化酶 (Gox) 能催化葡萄糖氧化反应，产生具有抗菌活性的 H_2O_2 和葡萄糖内酯，降低细菌生存环境的 pH 值，使大肠杆菌、金黄色葡萄球菌、单核细胞增生李斯特菌等食源性致病菌的生长受到抑制[76]。纤维二糖脱氢酶 (Cdh) 是另一种氧化还原酶，能产生有细胞毒性的 H_2O_2，抑制细菌细胞及生物被膜的生长[77]。

2.4.2 脂肪水解酶

脂质是许多细菌生物被膜中常见的基质成分，主要参与生物被膜表面的相互作用、附着和维持。从大蜡螟中分离出的脂肪酶对结核分枝杆菌 *Mycobacterium tuberculosis* H37Rv 具有杀灭作用，从牛胰腺中产生的脂肪酶可降解胞外基质，防止致病菌在海洋环境中形成生物被膜[78]。此外，有研究表明[79]，海洋杆菌属 PUMB02 *Oceanobacillus* spp.衍生的脂肪酶能够抑制食品加工环境中生物被膜的形成。

3 酶制剂与其他技术联合作用于生物被膜的研究

酶的专一性、高效性使其成为清除食源性致病菌生物被膜的绝佳手段，在实际应用过程中，许多研究者常将酶制剂与其他技术联合使用，包括多种酶制剂联合使用、酶制剂与化学试剂联合使用、酶制剂与物理杀菌方式联合使用等。这种联合作用的方式，拓展了酶制剂的多元应用功效，不仅能最大程度地清除生物被膜，还能有效地杀灭残留的致病菌，为食品质量安全控制提供了可靠的技术支撑。

3.1 多种酶制剂联合使用

不同酶制剂的作用靶点不同，如 QQ 酶抑制 QS 分子的积累、C-di-GMP 代谢酶催化调节 C-di-GMP 浓度、胞外基质水解酶水解胞外基质等，均能达到清除食源性致病菌生物被膜的目的。若将多种酶制剂联合使用，则能提高其清除细菌生物被膜的效率。Nguyen 等[80]研究了 DNase Ⅰ和蛋白酶 K 对单核细胞增生李斯特菌生物被膜的清除作用，当使用 100 μg/mL 的 DNase Ⅰ处理 72 h 的生物被膜时，生物被膜的残留量为 25%。若将 DNase Ⅰ与蛋白酶 K 联合使用，则能完全抑制生物被膜的形成。此外，Johansen 等[81]研究表明，氧化还原酶与多糖水解酶的组合可以清除生物被膜并产生杀菌活性。

3.2 酶制剂与化学杀菌技术联合使用

常规洗涤剂难以去除食品工业设备接口、缝隙等地方的生物被膜，若将酶制剂和洗涤剂联合使用，则可弥补传统洗涤剂的不足，提高洗涤剂的清洗效率。Walker 等[82]研究表明，通过酶制剂的预处理，有助于洗涤剂更好地清除生产线中生物被膜的污染。酶制剂的存在还能提高化学试剂的杀菌效率，Pedro 等[83]研究发现，当链霉蛋白酶或 DNase Ⅰ与苯扎氯铵 (Benzalkonium chloride，BAC) 一起使用时，可增强 BAC 的杀菌性能，有效降解单核细胞增生李斯特氏菌和大肠杆菌的混合生物被膜。笔者课题组研究发现，酸性电解水能有效清除副溶血性弧菌的生物被膜[84]，当其与 DNase Ⅰ联合使用时，可增强酸性电解水的清除作用[85]。

3.3 酶制剂与物理杀菌技术联合使用

酶制剂与物理杀菌方式结合是一种有效的清除食源性致病菌生物被膜方式。例如，Oulahal-Lagsir 等[86]利用淀粉葡萄糖苷酶结合超声波处理，成功清除了大肠杆菌在不锈钢表面的生物被膜。Oulahal 等[87]进一步研究了蛋白酶、木瓜蛋白酶、EDTA、超声波的联合处理方式，可有效清除不锈钢表面 75%的大肠杆菌生物被膜，而胰蛋白酶、溶菌酶、EDTA、超声波的联合处理方式，可清除不锈钢表面 100%的金黄色葡萄球菌生物被膜。

4 总结与展望

酶制剂能够靶向作用于 QS 信号分子、C-di-GMP、胞外多糖、胞外蛋白、eDNA 等胞外基质，从根本上清除食源性致病菌的生物被膜，而且因其具有特定的专一性和高效性，使致病菌很难产生相应的耐药性，因此被视为解决食源性致病菌生物被膜危害的重要技术手段之一[14]。本文系统地综述了不同种类的抗生物被膜酶制剂，为食源性致病菌生物被膜的有效控制提供了新的思路和视野。现阶段，酶制剂清除食源性致病菌生物被膜的研究已陆续开展，具有重要的科研价值和广阔的应用前景[12]。但是，抗生物被膜酶制剂在食品工业中的进一步开发与应用，仍存在不少技术难点和上升空间。因此，本文基于抗生物被膜酶制剂的研究现状，对其未来研究方向进行了以下三点展望。

4.1 提高抗生物被膜酶制剂的稳定性

酶的专一性和高效性在其清除食源性致病菌

生物被膜中发挥着重要作用，但酶的不稳定性可能会影响其在医疗卫生和食品工业中的应用[88]。因此，有效提升酶制剂的稳定性，对其实际的工业化应用至关重要。酶的定向进化技术获得了2018年诺贝尔化学奖[89]，该技术可极大地提升酶制剂的稳定性和催化效率。因此，可将该技术应用于抗生物被膜酶制剂的优化，在人工控制的特殊环境下，基于酶的三维结构，通过酶工程技术模拟自然进化机制，定向选择得到具有高稳定性、高清除效力的酶制剂，从而更好地推动抗食源性致病菌生物被膜酶制剂的开发。

4.2 开发基于酶制剂的抗生物被膜生物材料

抗生物被膜酶制剂的高效清除能力，使其在开发新型生物材料方面具有良好的应用前景。未来可合理运用固定化酶技术，将酶固定在玻璃、塑料、不锈钢等材料表面，从源头抑制生物被膜的形成，构建一种食源性致病菌生物被膜难以生长的新型生物材料。此外，还可将其与磁珠等纳米材料相结合，提高酶制剂的作用效果，扩大其在食品领域的应用范围。

4.3 促进抗生物被膜酶制剂的工业化

抗生物被膜酶制剂是一种绿色环保的技术，但是大多数酶制剂尚处于实验室研究阶段，仅证明了其高效的清除生物被膜能力，具体的工业化应用尚未完全开展。因此，应该利用基因工程技术筛选能够大量表达抗生物被膜酶制剂的高产菌株，提升酶的产量，增强酶的杀菌效果，提高其对食源性致病菌生物被膜的清除效率，推动抗被膜酶制剂在食品工业中的应用。

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