微生物学通报
Microbiology China
tongbao@im.ac.cn

Jan. 20, 2015, 42(1): 178−184

DOI: 10.13344/j.microbiol.china.140623

专论与综述

裂解酶治疗的研究进展与应用前景

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摘　要：多耐药病原细菌的不断出现和传播给公共医疗造成了严峻的威胁和挑战，开发新的抗菌分子迫在眉睫。噬菌体裂解酶来源于噬菌体，具有独特的进化和选择优势，不仅能高效快速的杀灭多耐药细菌，而且不易诱导细菌产生新的耐药性。本文对噬菌体裂解酶的结构和功能进行了简要的介绍，重点综述了裂解酶在抗细菌感染中近年的研究进展和应用前景。

关键词：噬菌体，噬菌体裂解酶，裂解酶治疗，病原细菌，抗生素耐药性

Research progress in lysin therapy

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Abstract: Treating infections caused by multi-drug resistant pathogenic bacteria is a challenge worldwide. It is critical to develop novel antimicrobial agents. Lysins are highly efficient peptidoglycan hydrolases which are encoded by bacteriophages. Because of their special advantages of co-evolution with bacteria and natural selectivity, lysins could kill multi-drug resistant bacteria with high efficacy and very low possibility of developing resistance. In the present review, the structures and functions of lysins are outlined, and the recent research progress in lysin therapy of bacterial infections is summarized.

Keywords: Phage, Bacteriophage lysin, Lysin therapy, Pathogenic bacterium, Antimicrobial resistance

近年来，多种超级耐药细菌的不断出现与传播给全球临床医学及细菌性传染病的治疗带来了严峻的挑战，加之新抗生素的研发面临着技术上以及商业上的障碍，近几年只有几种新抗生素上市。如果这种趋势继续，临床上有可能面临着无药可用的地步。人们现在越来越担心，是否我们将迈入因一个普通的细菌感染就会致命的后抗生素时代？如何有效应对细菌耐药性问题是当下亟待解决的难题。由于噬菌体对宿主细菌的裂解不受细菌耐药性的限制，噬菌体用于细菌感染的治疗受到了重新的考量与测试。但是，噬菌体治疗仍面临多种挑战，诸如噬菌体的细菌抗性、生物安全性、药代动力学

基金项目：国家 973 计划项目(No. 2012CB721102)；国家自然科学基金项目(No. 31400126)；中国科学院项目(No. KJZD-EW-L02)
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收稿日期：2014-08-16；接受日期：2014-09-12；优先数字出版日期(www.cnki.net)：2014-09-28

等。噬菌体裂解酶是噬菌体感染宿主晚期表达的一类肽聚糖水解酶，具有与细菌共同进化的选择优势和高效的裂解活性。裂解酶体外应用时能快速水解革兰氏阳性细菌的细胞壁，其作用不受细菌耐药性的影响，而且不易诱导细菌产生新的抗性。近年来建立的多种动物感染模型，初步体现了裂解酶用于耐药细菌感染治疗的潜力与优势。本文将综述噬菌体裂解酶在耐药细菌控制以及抗感染治疗中的研究进展，同时也将介绍革兰氏阴性细菌噬菌体裂解酶研究的最近进展。

1 噬菌体裂解酶

1.1 噬菌体裂解酶的发现

噬菌体裂解酶(Lysin)是双链 DNA 噬菌体感染宿主后晚期表达的一类肽聚糖水解酶。裂解酶在穴蛋白(Holin)的帮助下通过细胞膜孔洞抵达细胞壁上的肽聚糖靶点，对特定位点进行切割，直接造成细胞的破裂和瓦解，帮助子代噬菌体的释放。穴蛋白的作用是通过在细胞膜上形成低聚物对细胞膜进行穿孔，改变宿主细菌细胞膜的通透性，类似信号肽的功能。这种典型的穴蛋白-裂解酶裂解系统最早由 Young 等[1]提出，目前已得到普遍的认同。有些双链 DNA 噬菌体裂解酶不具备穴蛋白-裂解酶裂解系统。Loessner 等[2-3]研究发现，在蜡样芽胞杆菌(*Bacillus cereus*)的噬菌体裂解酶 TP21 和李斯特菌(*Listeria monocytogenes*)的噬菌体裂解酶 A511 中，裂解酶 N 端与信号肽序列同源性很高，具有与穴蛋白相似的功能，可以将自身引导至细胞壁靶点。

裂解酶的作用靶点是敏感细菌细胞壁上的肽聚糖链键，在体内借助穴蛋白的帮助而裂解靶细菌(自内裂解)，这是噬菌体和细菌共进化的结果。当裂解酶体外应用时，可以直接作用于敏感革兰氏阳性菌的细胞壁而导致细菌的快速裂解(自外裂解)[4]，而对革兰氏阴性菌没有作用。这种差异主要是由革兰氏阳性菌和革兰氏阴性菌细胞壁的构造不同决定的。革兰氏阴性菌细胞壁外的细胞膜阻碍了裂解酶与肽聚糖靶点的有效结合。裂解酶能自外裂解革兰氏阳性菌的发现开创了裂解酶用于耐药细菌控制与治疗的道路。

1.2 噬菌体裂解酶的结构

革兰氏阳性菌 DNA 噬菌体编码的裂解酶大小通常为 25−40 kD。一般来说，革兰氏阳性菌噬菌体裂解酶的结构相似[5-6]，具有一个决定该酶催化活性的 N-端催化功能域(Catalytic domain，CD)和一个决定细胞壁结合特异性的 C-端细胞壁结合功能域(Cell wall binding domain，CBD)，二者之间由一个小片段连接(图 1)。革兰氏阴性细菌噬菌体裂解酶的催化功能域一般位于 C 端，而细胞壁结合功能域则位于 N 端[7]。最近的研究表明有些裂解酶结构中还存在独立的芽孢识别功能域(Spore binding domain，SBD)，可以识别特定种属的细菌芽孢[8]。有些裂解酶拥有 2 个甚至 3 个不同的催化域[9]。裂解酶的细胞壁结合域可与宿主菌细胞壁上的特定基质(通常为糖类)高效、特异结合[10-11]，这是实现特异裂解靶细菌的前提[12]。基于序列比对发现，同一类裂解酶的 N-端催化域具有较高的同源性，而 C-端细胞壁结合域同源性低[12]。由于细胞裂解后溢出的裂解酶可能会杀死附近子代噬菌体的潜在宿主，阻碍噬菌体的进一步繁殖，这促使裂解酶不断进化其结合域，提高亲和力[12-13]，以限制游离裂解酶的释放。因此，有研究者认为裂解酶是一次性使用的酶类[14]，即裂解作用完成后，裂解酶不能有效地从细胞壁上解离下来。

根据裂解酶催化域活性的不同，可将其分为多种类型。催化域活性可体现为 N-乙酰-β-D-葡萄糖胺酶(N-acetyl-β-D-glucosaminidases)或 N-乙酰-β-D-胞壁酸酶(N-acetyl-β-D-muramidases)，或者内肽酶(Endopeptidases)，或者 N-乙酰胞壁酰-L-丙氨酸酰氨酶(N-acetylmuramoyl-L-alanine amidases)等(图 1)。N-乙酰-β-D-葡萄糖胺酶和 N-乙酰-β-D-胞壁酸酶二者都能作用于细菌细胞壁上的半糖；内肽酶水解肽侧链和交联桥之间的氨基或肽键连接；N-乙酰胞壁酰-L-丙氨酸酰氨酶能够水解连接糖链和肽基的酰胺键[1,15]。最近报道了一种具有 γ-D-谷氨酰胺酰-L-赖氨酸肽链内切酶活性的酶[16]，以及一个具有 D-丙氨酰-L-丙氨酸内肽酶活性的酶[17]。

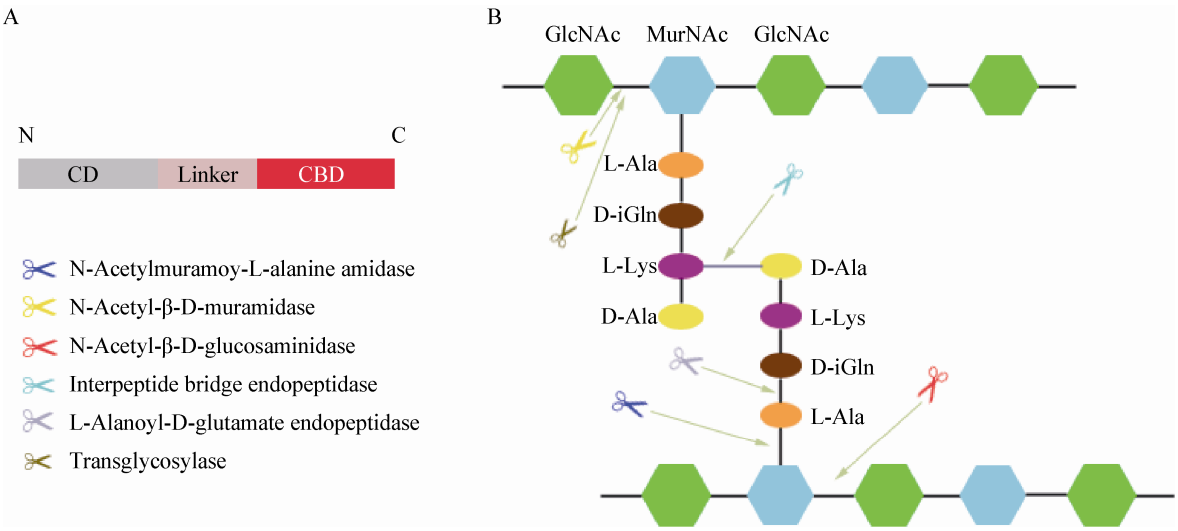

图 1 裂解酶的结构与水解位点

Figure 1 The schematic structure of lysins and their catalytic sites in peptidoglycan

注：A：噬菌体裂解酶的结构. 裂解酶一般包含一个 N 端的催化功能域(CD)和一个 C 端的细胞壁识别功能域(CBD)，二者由一个短片段连接(Linker). B：裂解酶在肽聚糖上的水解位点. 根据不同的结构，裂解酶可以表现出多种肽聚糖水解酶活性.

Note: A: The schematic structure of lysins. Most frequently, lysins displayed a typically modular structure of at least two distinct domains: an N-terminal CD and a C-terminal CBD. B: The cleavage sites of lysin in the peptidoglycan. The catalytic domains could display various enzymatic activities depending on their cleave sites.

2 裂解酶治疗

裂解酶最吸引人的地方在于裂解酶对病原细菌良好的杀灭能力，尤其是耐药细菌。裂解酶治疗正是在耐药细菌不断出现和传播的基础上提出来的。与传统的抗生素相比，裂解酶具有其独特的性质：(1) 裂解酶具有进化优势。裂解酶来自于噬菌体，噬菌体具有与宿主菌共同进化的特性。这是在长期的自然选择中保留下来的。(2) 裂解酶具有很强的特异性。裂解酶的细胞壁结合功能域只特异性的识别特定种属的细菌，这使得裂解酶具有很强的特异性。(3) 裂解酶具有高效的杀菌活力。裂解酶是释放子代噬菌体的“武器”，具有天然的高效性。(4) 裂解酶具有极低的产生耐药的可能性。迫于自然选择的压力，噬菌体只作用于宿主菌必不可少的保守部分，细菌很少能够产生抗性来逃避这种识别。

为了探索裂解酶治疗之路，研究人员做了大量的体内外试验，充分展现了裂解酶用于细菌感染治疗的巨大潜力[18-21]。截至目前，已经建立了大量的动物模型，包括皮肤感染模型[22]、粘膜去定殖模型[4,23-25]、阴道感染模型[26]、周身感染模型[27-31]、心内膜炎模型[32]、眼内炎模型[33]、脑膜炎模型[34]、肺炎感染模型[35-36]等。在这些动物模型中，噬菌体裂解酶均能快速杀灭靶细菌，在短时间内将细菌数量降低几个数量级，有些甚至低至无法检出。

例如，在一个皮肤感染模型中，Pastagia 等用嵌合裂解酶 ClyS 能有效地消灭小鼠烧伤皮肤感染的 MRSA，使其降低了 3 个数量级[22]。作用效果比外用抗感染药莫匹罗星好(莫匹罗星作用后只能降低 2 个数量级)，而且细菌对 ClyS 产生耐药性的可能性远低于莫匹罗星。

在一个粘膜去定殖模型中，Loeffler 等[35]利用肺炎链球菌噬菌体裂解酶 Pal 清除小鼠鼻腔中的肺炎链球菌。Pal 在体外能高效裂解 15 种常见类型的肺炎链球菌，包括青霉素抗性菌株。一次给药 5 h 后，能将小鼠鼻腔定殖的肺炎链球菌降到无法检测到的水平。Pal 在杀灭靶细菌的同时对口腔中的其他微生物没有影响，而且也没有耐药细菌的产生。

在一个阴道感染模型中，Cheng 等[26]利用裂解酶 PlyGBS 降低了小鼠阴道定殖的 B 族链球菌。研究者指出 PlyGBS 具有高效清除产前妇女阴道中的 B 族链球菌的潜力，可以降低新生儿感染的几率。而通常的做法是对产前妇女使用抗生素来预防和降低这种感染的风险。PlyGBS 具有比抗生素更好的效果，而且不存在过敏现象和产生细菌耐药性等隐患。

在一个周身感染模型中，Gu 等[28]在小鼠感染 1 h 后一次性腹腔注射 50 μg 裂解酶 LysGH15 能有效提高 2 倍于致死剂量的 MRSA 感染的小鼠的存活率。在没有裂解酶保护的实验组中，在感染 3.5 h 后，小鼠血液中细菌浓度为 10^7 CFU/mL。而在有裂解酶保护的实验组中，在感染 3.5 h 后小鼠血液中细菌浓度为 10^4 CFU/mL，而在几天后，细菌数量低至无法检出。

与噬菌体治疗相比，裂解酶治疗有其独特的优势。譬如，裂解酶是蛋白质性质，可以建立明确的质控标准；裂解酶既可以从烈性噬菌体中分离得到，也可以从温和噬菌体中分离得到；可以方便地进行设计和改造等[37]。因此，裂解酶治疗得到了国内外许多学者的支持和倡导，各方面的研究均体现了裂解酶用于耐药细菌感染控制与治疗的潜力和发展空间。令人鼓舞的是，近年来有些裂解酶已经进入到了临床前的试验阶段(例如 ClyS，P128 等)，而在美国已出现了开发裂解酶产品的高科技公司(如 ContraFect)。ClyS 是一个人工设计的嵌合裂解酶，由噬菌体 Twort 裂解酶 plyTW 的催化域和噬菌体 phNM3 裂解酶的细胞壁结合域拼接而成。这种设计增强了 ClyS 的水溶性和裂解活性，为临床试验提供了保障。但有关该裂解酶临床试验的结果目前还没有正式对外公布。值得一提的是，一个针对葡萄球菌的裂解酶 P128 已经进入到临床二期(http://www.gangagen.com/research.html)。从网站上了解到的情况来看，该裂解酶临床试验的效果很好。表 1 简要列举了近年来裂解酶治疗研究的一些实践例子。

表 1 裂解酶治疗实例(小鼠模型)
Table 1 Lysins that have been tested in mouse models

Lysin	Phage source	Bacteria	Murine sepsis challenge	Reference
Cpl-1	Cp1	*S. pneumoniae*	Nasopharynx, mucosa, bloodstream, aortic valve	[31-32,34-35,38-40]
Pal	Dp-1	*S. pneumoniae*	Nasopharynx, oropharynx, intraperitoneal	[31,35]
C1	C1	*S. pyogenes*	Upper respiratory colonization	[4]
PlyG	Gamma	*B. anthracis*	Intraperitoneal, bacteremia	[41]
PlyPH		*B. anthracis*	Intraperitoneal, bacteremia	[42]
PlyV12	Phi1	*E. faecalis* and *E. faecium*	Intraperitoneal, bacteremia	[43]
MV-L	MR11	*S. aureus*	Intraperitoneal, bacteremia	[44]
PlyGBS	NCTC 11261	GBS	Upper respiratory colonization, vaginal colonization	[26]
Lysin of phage ENB6	ENB6	Vancomycin-resistant *E. faecium*	Intraperitoneal, bacteremia	[45]
Pal and Cpl-1	Dp-1 and Cp-1	*S. pneumonia*	Intraperitoneal, bacteremia	[31,38]
LytA		*S. pneumoniae*	Peritonitis-sepsis	[46]
PlyC	phiC1	*S. pyogenes*	Oral mucosa, nasal mucosa	[4,47]
ClyS		MRSA	Nasal colonization, bacteremia	[29]
ClyS		MRSA and MSSA	Skin colonization	[22]
LysGH15	GH15	MRSA	Intraperitoneal, bacteremia	[28,48]
PlySs2	*S. suis* phage	*S. pyogenes* and MRSA	Intraperitoneal, bacteremia	[49]
SAL-1	SAL	*S. aureus*	Intraperitoneal, bacteremia	[50]
CF-301	*S. suis* phage	*S. aureus*	Intraperitoneal, bacteremia	[51]
ClyH		MRSA	Intraperitoneal, bacteremia	[52]
PlyPy	*S. pyogenes*	Streptococci	Intraperitoneal, bacteremia	[17]

3 阴性菌裂解酶

根据裂解酶的作用原理，裂解酶从外至内裂解细菌时只能作用于革兰氏阳性细菌。因革兰氏阴性细菌细胞壁外面的细胞膜能阻挡裂解酶与肽聚糖靶点的有效结合，裂解酶从外加入时不能裂解革兰氏阴性细菌。因此，不能在体外直接裂解革兰氏阴性细菌是裂解酶应用中的一个重大挑战。

但是最近一些研究表明，该挑战有可能通过一些设计而得到解决，显示了良好的前景。如 Buchanan 及其研究团队利用耶尔森氏菌细胞膜上的离子通道，将鼠疫菌素上能特异识别离子通道的序列与 T4 溶酶体融合，所得到的重组蛋白能借助离子通道通过耶尔森氏菌的细胞膜，导致耶尔森氏菌的死亡。而且对大肠杆菌也有明显的杀灭效果[53-55]。最近的其他研究表明由抗菌肽与裂解酶构成的融合蛋白可以在体外直接裂解铜绿假单胞菌[56]。该研究将抗菌肽 SMAP-29 融合在裂解酶 KZ144 的 N 端，通过大肠杆菌表达纯化后，SMAP-29-KZ144 能够在其 N 端抗菌肽的帮助下通过细胞膜抵达细胞壁上的肽聚糖靶点，切割肽聚糖链键杀灭阴性菌铜绿假单胞菌。这种设计充分利用了抗菌肽的细胞膜破坏功能与裂解酶的肽聚糖水解功能，为多耐药阴性细菌的控制与治疗提供了新的思路。尤其值得指出的是，沿用这种思路设计的系列抗菌肽裂解酶对铜绿假单胞菌的体内杀灭效果已经在线虫感染模型中得到了证实[57]。

4 裂解酶存在的问题

首先，有研究指出天然裂解酶在大肠杆菌中表达时有些对表达菌株具有明显的毒性，蛋白表达往往以包涵体的形式存在[29,58-59]。很难得到有高活力的裂解酶，这可能与裂解酶胞壁质水解酶的活力有关。该问题有可能通过构建合适的嵌合裂解酶(组合不同来源裂解酶的催化域和结合域)的方式进行解决。

其次，裂解酶本质上是蛋白质，进入机体后易受到蛋白酶的攻击，而具有较短的半衰期。这一问题有可能会制约裂解酶用于体内治疗的有效工作浓度和时间。和噬菌体不同，后者具有自我繁殖的能力，一次给药就可能产生持续的治疗效果。

此外，裂解酶还存在一个比较普遍的问题：可溶性差。裂解酶的 C 端是用来特异性结合宿主细菌的，该部分在结构上通常具有较强的疏水性和多个重复的跨膜区[60]。这使得裂解酶在水溶液中的溶解性较差。在体外应用时还需要摸索合适的缓冲体系。

5 展望

噬菌体裂解酶具有抗生素与噬菌体所没有的优势：蛋白质性质、进化优势、高效的杀菌活性、清楚的结构、易于人工设计和改造、不易产生耐药性等。这些特点使得裂解酶具有用于临床耐药细菌控制与治疗的巨大潜力。目前的许多基础研究也支持这个论断。但是其对蛋白酶的敏感性、半衰期、可溶性、代谢途径、组织渗透性、免疫原性以及细胞毒性等因素仍然需要进一步的深入研究和寻找解决方法。譬如，裂解酶的大规模生产可能受到其可溶性差等因素的制约，而较短的半衰期对裂解酶的剂型和给药时间提出了新的要求。

参 考 文 献

[1] Young R. Bacteriophage lysis: mechanism and regulation[J]. Microbiology and Molecular Biology Reviews, 1992, 56(3): 430-481
[2] Loessner MJ, Maier SK, Daubek-puza H, et al. Three *Bacillus cereus* bacteriophage endolysins are unrelated but reveal high homology to cell wall hydrolases from different bacilli[J]. Journal of Bacteriology, 1997, 179(9): 2845-2851
[3] Loessner MJ, Wendlinger G, Scherer S. Heterogeneous endolysins in *Listeria monocytogenes* bacteriophages: a new class of enzymes and evidence for conserved holin genes within the siphoviral lysis cassettes[J]. Molecular Microbiology, 1995, 16(6): 1231-1241
[4] Nelson D, Loomis L, Fischetti VA. Prevention and elimination of upper respiratory colonization of mice by group A streptococci by using a bacteriophage lytic enzyme[J]. Proceedings of the National Academy of Sciences of the United States of America, 2001, 98(7): 4107-4112
[5] Diaz E, Lopez R, Garcia JL. Chimeric phage-bacterial enzymes: a clue to the modular evolution of genes[J]. Proceedings of the National Academy of Sciences of the United States of America, 1990, 87(20): 8125-8129
[6] Garcia P, Garcia JL, Garcia E, et al. Modular organization of the lytic enzymes of *Streptococcus pneumoniae* and its bacteriophages[J]. Gene, 1990, 86(1): 81-88
[7] Briers Y, Volckaert G, Cornelissen A, et al. Muralytic activity

and modular structure of the endolysins of *Pseudomonas aeruginosa* bacteriophages phiKZ and EL[J]. Molecular Microbiology, 2007, 65(5): 1334-1344
[8] Yang H, Wang DB, Dong Q, et al. Existence of separate domains in lysin PlyG for recognizing *Bacillus anthracis* spores and vegetative cells[J]. Antimicrobial Agents and Chemotherapy, 2012, 56(10): 5031-5039
[9] Navarre WW, Ton-That H, Faull KF, et al. Multiple enzymatic activities of the murein hydrolase from staphylococcal phage phi11. Identification of a D-alanyl-glycine endopeptidase activity[J]. Journal of Biological Chemistry, 1999, 274(22): 15847-15856
[10] Lopez R, Garcia E, Garcia P, et al. The pneumococcal cell wall degrading enzymes: a modular design to create new lysins?[J]. Microbial Drug Resistance, 1997, 3(2): 199-211
[11] Garcia E, Garcia JL, Garcia P, et al. Molecular evolution of lytic enzymes of *Streptococcus pneumoniae* and its bacteriophages[J]. Proceedings of the National Academy of Sciences of the United States of America, 1988, 85(3): 914-918
[12] Thouand G, Vachon P, Liu S, et al. Optimization and validation of a simple method using P22::luxAB bacteriophage for rapid detection of *Salmonella enterica* serotypes A, B, and D in poultry samples[J]. Journal of Food Protection, 2008, 71(2): 380-385
[13] Loessner MJ, Kramer K, Ebel F, et al. C-terminal domains of *Listeria monocytogenes* bacteriophage murein hydrolases determine specific recognition and high-affinity binding to bacterial cell wall carbohydrates[J]. Molecular Microbiology, 2002, 44(2): 335-349
[14] Fischetti VA. Bacteriophage lysins as effective antibacterials[J]. Current Opinion in Microbiology, 2008, 11(5): 393-400
[15] Loessner MJ. Bacteriophage endolysins—current state of research and applications[J]. Current Opinion in Microbiology, 2005, 8(4): 480-487
[16] Pritchard DG, Dong S, Kirk MC, et al. LambdaSa1 and LambdaSa2 prophage lysins of *Streptococcus agalactiae*[J]. Applied and Environmental Microbiology, 2007, 73(22): 7150-7154
[17] Lood R, Raz A, Molina H, et al. A highly active and negatively charged *Streptococcus pyogenes* lysin with a rare D-alanyl-L-alanine endopeptidase activity protects mice against streptococcal bacteremia[J]. Antimicrobial Agents and Chemotherapy, 2014, 58(6): 3073-3084
[18] Fischetti VA. Bacteriophage endolysins: a novel anti-infective to control Gram-positive pathogens[J]. International Journal of Medical Microbiology, 2010, 300(6): 357-362
[19] Fenton M, Ross P, Mcauliffe O, et al. Recombinant bacteriophage lysins as antibacterials[J]. Bioengineered Bugs, 2010, 1(1): 9-16
[20] Borysowski J, Weber-dabrowska B, Gorski A. Bacteriophage endolysins as a novel class of antibacterial agents[J]. Experimental Biology and Medicine (Maywood), 2006, 231(4): 366-377
[21] Schmelcher M, Donovan DM, Loessner MJ. Bacteriophage endolysins as novel antimicrobials[J]. Future Microbiology, 2012, 7(10): 1147-1171
[22] Pastagia M, Euler C, Chahales P, et al. A novel chimeric lysin shows superiority to mupirocin for skin decolonization of methicillin-resistant and -sensitive *Staphylococcus aureus* strains[J]. Antimicrobial Agents and Chemotherapy, 2011, 55(2): 738-744
[23] Fischetti VA. Novel method to control pathogenic bacteria on human mucous membranes[J]. Annals of the New York Academy of Sciences, 2003, 987: 207-214
[24] Kiser KB, Cantey-kiser JM, Lee JC. Development and characterization of a *Staphylococcus aureus* nasal colonization model in mice[J]. Infection and Immunity, 1999, 67(10): 5001-5006
[25] Kluytmans J, Van BA, Verbrugh H. Nasal carriage of *Staphylococcus aureus*: epidemiology, underlying mechanisms, and associated risks[J]. Clinical Microbiology Reviews, 1997, 10(3): 505-520
[26] Cheng Q, Nelson D, Zhu S, et al. Removal of group B streptococci colonizing the vagina and oropharynx of mice with a bacteriophage lytic enzyme[J]. Antimicrobial Agents and Chemotherapy, 2005, 49(1): 111-117
[27] Schmelcher M, Powell AM, Becker SC, et al. Chimeric phage lysins act synergistically with lysostaphin to kill mastitis-causing *Staphylococcus aureus* in murine mammary glands[J]. Applied and Environmental Microbiology, 2012, 78(7): 2297-2305
[28] Gu J, Xu W, Lei L, et al. LysGH15, a novel bacteriophage lysin, protects a murine bacteremia model efficiently against lethal methicillin-resistant *Staphylococcus aureus* infection[J]. Journal of Clinical Microbiology, 2011, 49(1): 111-117
[29] Daniel A, Euler C, Collin M, et al. Synergism between a novel chimeric lysin and oxacillin protects against infection by methicillin-resistant *Staphylococcus aureus*[J]. Antimicrobial Agents and Chemotherapy, 2010, 54(4): 1603-1612
[30] Manoharadas S, Witte A, Blasi U. Antimicrobial activity of a chimeric enzybiotic towards *Staphylococcus aureus*[J]. Journal of Biotechnology, 2009, 139(1): 118-123
[31] Jado I, Lopez R, Garcia E, et al. Phage lytic enzymes as therapy for antibiotic-resistant *Streptococcus pneumoniae* infection in a murine sepsis model[J]. Journal of Antimicrobial Chemotherapy, 2003, 52(6): 967-973
[32] Entenza JM, Loeffler JM, Grandgirard D, et al. Therapeutic effects of bacteriophage Cpl-1 lysin against *Streptococcus pneumoniae* endocarditis in rats[J]. Antimicrobial Agents and Chemotherapy, 2005, 49(11): 4789-4792
[33] Singh PK, Donovan DM, Kumar A. Intravitreal injection of the chimeric phage endolysin Ply187 protects mice from *Staphylococcus aureus* endophthalmitis[J]. Antimicrobial Agents and Chemotherapy, 2014, 58(8): 4621-4629
[34] Grandgirard D, Loeffler JM, Fischetti VA, et al. Phage lytic enzyme Cpl-1 for antibacterial therapy in experimental *pneumococcal meningitis*[J]. Journal of Infectious Diseases, 2008, 197(11): 1519-1522
[35] Loeffler JM, Nelson D, Fischetti VA. Rapid killing of *Streptococcus pneumoniae* with a bacteriophage cell wall hydrolase[J]. Science, 2001, 294(5549): 2170-2172
[36] Witzenrath M, Schmeck B, Doehn JM, et al. Systemic use of the endolysin Cpl-1 rescues mice with fatal *Pneumococcal pneumonia*[J]. Critical Care Medicine, 2009, 37(2): 642-649
[37] O'flaherty S, Ross RP, Coffey A. Bacteriophage and their lysins for elimination of infectious bacteria[J]. FEMS Microbiology Reviews, 2009, 33(4): 801-819
[38] Loeffler JM, Fischetti VA. Synergistic lethal effect of a combination of phage lytic enzymes with different activities on penicillin-sensitive and -resistant *Streptococcus pneumoniae* strains[J]. Antimicrobial Agents and Chemotherapy, 2003, 47(1): 375-377
[39] Loeffler JM, Djurkovic S, Fischetti VA. Phage lytic enzyme Cpl-1 as a novel antimicrobial for pneumococcal bacteremia[J]. Infection and Immunity, 2003, 71(11): 6199-6204
[40] Mccullers JA, Karlstrom A, Iverson AR, et al. Novel strategy to prevent otitis media caused by colonizing *Streptococcus pneumoniae*[J]. PLoS Pathogens, 2007, 3(3): e28
[41] Schuch R, Nelson D, Fischetti VA. A bacteriolytic agent that detects and kills *Bacillus anthracis*[J]. Nature, 2002, 418(6900): 884-889
[42] Yoong P, Schuch R, Nelson D, et al. PlyPH, a bacteriolytic enzyme with a broad pH range of activity and lytic action against *Bacillus anthracis*[J]. Journal of Bacteriology, 2006, 188(7): 2711-2714
[43] Yoong P, Schuch R, Nelson D, et al. Identification of a broadly

active phage lytic enzyme with lethal activity against antibiotic-resistant *Enterococcus faecalis* and *Enterococcus faecium*[J]. Journal of Bacteriology, 2004, 186(14): 4808-4812
[44] Rashel M, Uchiyama J, Ujihara T, et al. Efficient elimination of multidrug-resistant *Staphylococcus aureus* by cloned lysin derived from bacteriophage phi MR11[J]. Journal of Infectious Diseases, 2007, 196(8): 1237-1247
[45] Biswas B, Adhya S, Washart P, et al. Bacteriophage therapy rescues mice bacteremic from a clinical isolate of vancomycin-resistant *Enterococcus faecium*[J]. Infection and Immunity, 2002, 70(1): 204-210
[46] Rodriguez-cerrato V, Garcia P, Huelves L, et al. Pneumococcal LytA autolysin, a potent therapeutic agent in experimental peritonitis-sepsis caused by highly beta-lactam-resistant *Streptococcus pneumoniae*[J]. Antimicrobial Agents and Chemotherapy, 2007, 51(9): 3371-3373
[47] Cheng Q, Fischetti VA. Mutagenesis of a bacteriophage lytic enzyme PlyGBS significantly increases its antibacterial activity against group B streptococci[J]. Applied Microbiology and Biotechnology, 2007, 74(6): 1284-1291
[48] Gu J, Zuo J, Lei L, et al. LysGH15 reduces the inflammation caused by lethal methicillin-resistant *Staphylococcus aureus* infection in mice[J]. Bioengineered Bugs, 2011, 2(2): 96-99
[49] Gilmer DB, Schmitz JE, Euler CW, et al. Novel bacteriophage lysin with broad lytic activity protects against mixed infection by *Streptococcus pyogenes* and methicillin-resistant *Staphylococcus aureus*[J]. Antimicrobial Agents and Chemotherapy, 2013, 57(6): 2743-2750
[50] Jun SY, Jung GM, Yoon SJ, et al. Antibacterial properties of a pre-formulated recombinant phage endolysin, SAL-1[J]. International Journal of Antimicrobial Agents, 2013, 41(2): 156-161
[51] Schuch R, Lee HM, Schneider BC, et al. Combination therapy with lysin CF-301 and antibiotic is superior to antibiotic alone for treating methicillin-resistant *Staphylococcus aureus*-induced murine bacteremia[J]. Journal of Infectious Diseases, 2014, 209(1): 1469-1478
[52] Yang H, Zhang Y, Yu J, et al. Novel chimeric lysin with high-level antimicrobial activity against methicillin-resistant *Staphylococcus aureus in vitro* and *in vivo*[J]. Antimicrobial Agents and Chemotherapy, 2014, 58(1): 536-542
[53] Lukacik P, Barnard TJ, Buchanan SK. Using a bacteriocin structure to engineer a phage lysin that targets Yersinia pestis[J]. Biochemical Society Transactions, 2012, 40(6): 1503-1506
[54] Lukacik P, Barnard TJ, Keller PW, et al. Structural engineering of a phage lysin that targets gram-negative pathogens[J]. Proceedings of the National Academy of Sciences of the United States of America, 2012, 109(25): 9857-9862
[55] Lukacik P, Barnard TJ, Hinnebusch BJ, et al. Specific targeting and killing of Gram-negative pathogens with an engineered phage lytic enzyme[J]. Virulence, 2013, 4(1): 90-91
[56] Briers Y, Walmagh M, Grymonprez B, et al. Art-175 is a highly efficient antibacterial against multidrug-resistant strains and persisters of *Pseudomonas aeruginosa*[J]. Antimicrobial Agents and Chemotherapy, 2014, 58(7): 3774-3784
[57] Briers Y, Walmagh M, Van PV, et al. Engineered endolysin-based "artilysins" to combat multidrug-resistant gram-negative pathogens[J]. MBio, 2014, 5(4): e01379-14
[58] Becker SC, Foster-frey J, Donovan DM. The phage K lytic enzyme LysK and lysostaphin act synergistically to kill MRSA[J]. FEMS Microbiology Letters, 2008, 287(2): 185-191
[59] O'flaherty S, Coffey A, Meaney W, et al. The recombinant phage lysin LysK has a broad spectrum of lytic activity against clinically relevant staphylococci, including methicillin-resistant *Staphylococcus aureus*[J]. Journal of Bacteriology, 2005, 187(20): 7161-7164
[60] Hermoso JA, Monterroso B, Albert A, et al. Structural basis for selective recognition of pneumococcal cell wall by modular endolysin from phage Cp-1[J]. Structure, 2003, 11(10): 1239-1249