中国科学: 生命科学　　2023 年　第 53 卷　第 5 期: 698 ~ 710
SCIENTIA SINICA Vitae　　lifecn.scichina.com
《中国科学》杂志社 SCIENCE CHINA PRESS
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评 述　　中国科学院学部　科学与技术前沿论坛　微生物群系与大健康专辑

噬菌体微生态疗法与一体化健康: 现状、挑战与机遇

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收稿日期: 2021-11-04; 接受日期: 2021-12-08; 网络版发表日期: 2022-06-13
国家自然科学基金(批准号: 42007025, 42090060, 41922053)、江苏省自然科学基金(批准号: BK20200533)和中央高校基本科研业务费专项资金(批准号: KJQN202116)资助

摘要　在全球化和气候变化的背景下, 病原细菌跨区域乃至全球性的广泛传播和危害加剧成为当前农业、环境和健康领域的重大共性难题, 威胁土壤-植物-动物-环境一体化健康(One Health). 滥用农药和抗生素所带来的生态环境和卫生安全风险成为国际共识, 靶向阻控病原细菌的噬菌体疗法重新引起人们的重视. 本文梳理了农业种植和养殖领域中应用噬菌体阻控病原细菌的现状; 从改变病原细菌的生态与进化, 调控土著微生物群落结构与功能以及激发病原细菌真核宿主的免疫系统等三方面总结噬菌体疗法的微生态机制; 从病原细菌基因、生理和生态型的变异性, 噬菌体与病原菌互作博弈的局限性, 噬菌体生产应用的复杂性等方面论述噬菌体疗法发展应用的挑战; 从一体化健康的需求、噬菌体疗法技术突破和多技术协同等方面探讨噬菌体疗法在一体化健康中的发展机遇.

关键词　病原细菌, 微生态, 微生物群系, 噬菌体疗法, 一体化健康

以牺牲环境、滥用农药和抗生素等为特征的集约化农业的快速发展导致了土壤退化、资源利用率低和环境恶化等严重问题. 在全球化进程和气候变化加剧的背景下, 由病原细菌引起的农业植物健康、动物健康和环境健康问题呈现全球大流行趋势, 严重威胁着土壤-植物-动物-环境一体化健康[1,2]. 一体化健康是涉及人类、动物、环境卫生健康的一种跨学科跨地域协作和交流的新策略, 致力于共同促进人和动物健康, 维护和改善生态环境[3]. 一些植物病原菌、食源性条件致病菌(opportunistic pathogen)的扩散传播, 在造成作

引用格式:　韦中, 王孝芳, 马迎飞, 等. 噬菌体微生态疗法与一体化健康: 现状、挑战与机遇. 中国科学: 生命科学, 2023, 53: 698–710
Wei Z, Wang X F, Ma Y F, et al. Phage therapy for One Health approach: current status, challenges and opportunities (in Chinese). Sci Sin Vitae, 2023, 53: 698–710, doi: 10.1360/SSV-2021-0402

物减产、食品质量下降的同时, 不断引发人畜共患病, 严重威胁了人类公共卫生安全. 抗生素和化学农药等因其见效快被广泛用于抑制农业种植、养殖等领域的病原细菌, 但不合理利用会产生较高的生态和人群健康风险. 首先, 抗生素滥用显著增加病原菌的耐药性, 进而加剧感染风险和防控难度, 例如, 耐甲氧西林金黄色葡萄球菌(*Staphylococcus aureus*)[4]和耐万古霉素粪肠球菌(*Enterococcus faecalis*)[5]等已成为一体化健康的巨大威胁. 其次, 在病原菌与其宿主互作的热点区还存在大量土著微生物群落, 这些微生物之间通过复杂的协同和拮抗作用形成稳态[6~8], 在抵御病原菌侵入和感染宿主组织中发挥重要作用. 然而, 广谱杀菌的抗生素和化学农药等在抑制病原菌的同时, 也严重破坏了土著微生物群落的互作稳态, 进而损害宿主健康. 为实现一体化健康, 急需靶向性强且环境友好的新措施以高效阻控农业-环境系统中病原细菌的传播.

噬菌体是专性侵染细菌的一类病毒, 在环境中普遍存在. 据估计, 地球生物圈中噬菌体的数量高达10^{31}个. 利用噬菌体阻控病原细菌的治疗手段被称为“噬菌体疗法”, 被认为是一种可替代抗生素的绿色生态技术. 简单来说, 噬菌体疗法具有以下优势: 噬菌体的宿主特异性强, 可以识别特定病原菌, 对环境扰动小; 利用宿主进行增殖, 高效裂解病原菌; 与宿主协同进化, 动态阻控病原菌. 自噬菌体被发现以来, 从首次治疗人类痢疾(1919年), 到治疗葡萄球菌引起的皮肤感染(1921年), 噬菌体疗法不断发展, 2007年欧洲食品安全局(European Food Safety Authority, EFSA)、美国食品和药物监督管理局(Food and Drug Administration, FDA)批准噬菌体鸡尾酒疗法(多噬菌体组合)用于抑制农业食品中的病原菌. 目前噬菌体疗法已广泛应用于临床医疗、食品、畜牧业、水产养殖和种植业等领域[9,10](图1). 噬菌体疗法的应用不仅减少了抗生素的使用, 还为食品安全提供了保障, 在推动一体化健康的过程中发挥重要作用, 但目前大多数研究还是关注噬菌体与靶细菌的互作机制, 缺少对微生态效应和作用机制的系统总结和认知. 本文主要梳理了当前噬菌体在农业种植和养殖领域中阻控病原细菌的应用现状; 从改变病原细菌的生态与进化, 调控土著微生物群落结构与功能以及激发病原细菌真核宿主的免疫系统等三方面总结噬菌体疗法的微生态机制; 从病原细菌基因、生理和生态型的变异性, 噬菌体与病原菌互作博弈的局限性, 噬菌体生产应用的复杂性等方面论述噬菌体疗法发展应用的挑战; 从一体化健康的需求、噬菌体疗法技术突破和多技术协同等方面探讨噬菌体疗法在一体化健康中的发展机遇.

1　噬菌体在一体化健康领域的应用现状

1.1　噬菌体疗法在农业种植领域的应用现状

噬菌体疗法在解决由病原细菌侵染引起的作物健康问题方面已有不少尝试. 1924年, 研究人员发现白菜滤液中的噬菌体类物质能够防止由黄单胞菌引起的白菜腐烂[12]. 随后, 该物质又被广泛应用于防治由青枯菌(*Ralstonia solanacearum*)、欧文氏菌(*Erwinia pyrifoliae*)、丁香假单胞菌(*Pseudomonas syringae* pv. *Actinidiae*)和野油菜黄单胞菌(*Xanthomonas campestris* pv. *campestris*)等引起的茄科作物青枯病、猕猴桃细菌性溃疡病、果树火疫病、柑橘斑点病、水稻叶枯病以及土豆洋葱软腐病和黑胫病等(表1), 均具有一定的防控效果. 2005年, 美国环境保护局首次批准了防治由野油菜黄单胞菌(*Xanthomonas campestris*)和丁香假单胞菌引起的番茄和辣椒的细菌斑点病的噬菌体产品; 2011年, 美国环境保护局批准OmniLytics公司的生物农药AgriPhage^{TM}用于防治番茄的溃疡病. 目前国内虽然还没有针对植物病害的噬菌体产品上市, 但多个研究单位针对不同的植物病害收集了大量的噬菌体资源, 且具有较好的病害防控效果. 例如, 近年来, 南京农业大学微生态与根际健康实验室初步建立了全国土传青枯菌专性噬菌体资源库, 并探究精准靶向土传青枯菌的噬菌体疗法的微生态调控原理和技术体系.

1.2　噬菌体疗法在畜牧和水产养殖业的应用现状

抗生素的耐药性及药物残留等问题已威胁到食品安全和人类健康, 噬菌体疗法作为抗生素的替代品引起广泛的关注. 噬菌体制剂的应用对减少畜禽肠道微生态的破坏以及抗生素抗性风险具有重要意义. 目前已有针对多种人畜致病菌, 如大肠杆菌(*Escherichia coli*)、沙门氏菌(*Salmonella*)、肺炎克雷伯菌(*Klebsiella pneumoniae*)、金黄色葡萄球菌、铜绿假单胞菌(*Pseudomonas aeruginosa*)、副溶血弧菌(*Vibrio parahaemolyticus*)和单核细胞增生李斯特菌(*Listeria monocytogenes*)等的噬菌体疗法报道(表1). Hosseindoust等

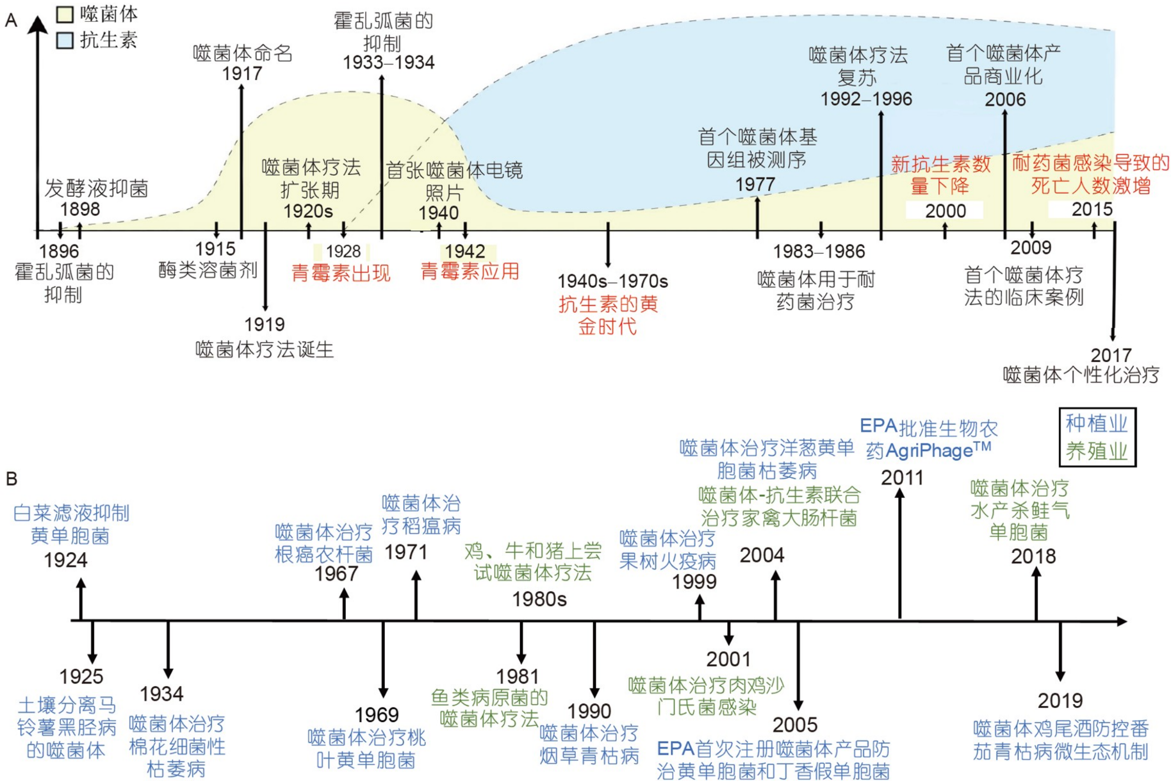

图 1 噬菌体疗法的研究进展. A: 噬菌体和抗生素研究历史上主要事件时间表(修改自文献[11]); B: 农业种植和养殖领域中噬菌体疗法的关键事件

Figure 1 Research progress of phage therapy. A: The timeline of major events in the history of research on phage and antibiotics (modified version from the work by Gordillo Altamirano and Barr [11]); B: the major events of phage therapy in agricultural cultivation and aquaculture

表 1 噬菌体疗法防控农业病原细菌的概况

Table 1 Experimental studies using phage to control bacterial pathogen in agriculture

分类	宿主植物	病原菌或疾病
植物	番茄	青枯菌(*Ralstonia solanacearum*)[13,14]
	猕猴桃、樱桃	丁香假单胞菌(*Pseudomonas syringae*)[15~19]
	苹果、梨	欧文氏菌(*Erwinia amylovora*)[20~23]
	水稻	黄单胞菌(*Xanthomonas oryzae* pv. *Oryzae*)[24~26]
	土豆、洋葱	果胶杆菌属(*Pectobacterium* spp./*Dickeya solani*)[27~30]
动物	牛	金黄色葡萄球菌(*Staphylococcus aureus*)[31,32]
	鸡	沙门氏菌(*Salmonella*)[33]、弯曲杆菌(*Campylobacter*)[34]
	猪	沙门氏菌(*Salmonella*)[35,36]、肠杆菌(*Enterobacter*)[37]
	水产	副溶血弧菌(*Vibrio parahaemolyticus*)[38]、哈维氏弧菌(*Vibrio harveyi*)[39]、变形假单胞菌(*Pseudomonas plecoglossicida*)[40]、杀鲑气单胞菌(*Aeromonas salmonicida*)[41]、溶藻弧菌(*Vibrio alginolyticus*)[42]

人[43]在仔猪饲料中添加大肠杆菌噬菌体(10^9 PFU/g), 连续饲喂35天, 有效减少了仔猪回肠和盲肠梭状杆菌(*Clostridium* spp.)及回肠中大肠杆菌数量. Miller等人[44]的研究表明, 产气荚膜梭菌噬菌体鸡尾酒效果优于产气荚膜梭菌类毒素疫苗, 可将肉鸡坏死性肠炎的死亡率降低92%. Dubey等人[45]向副溶血弧菌感染后

的虾水体中以感染复数(multiplicity of infection, MOI)=100加入单株副溶血弧菌噬菌体，仅1小时虾体内的副溶血弧菌载量就降低了78.1%．2013年由美国Intralytix公司研发的针对食源性沙门氏菌的噬菌体制剂SalmoFresh™获FDA批准上市，归类为公认安全级产品(Generally Regarded As Safe, GRAS). 目前，国内如南京农业大学、中国科学院深圳先进技术研究院、大连理工大学、江苏省农业科学院等多家研究机构研发噬菌体相关技术与产品，取得很大进展．

土壤和水是农业种植和养殖的主要载体，作为开放的系统，与外界进行着频繁的物质和能量交换．随着污水灌溉和畜禽粪便肥料施用等人为活动，病原细菌进一步扩散到土壤以及水体等农业环境中并大量增殖[46]．如不加控制，这些病原细菌会再次随水体传播至其他区域，侵染植物或伴随食品加工等途径进入食物链，导致动物和人患病，威胁农业生产安全和人类健康[47]．以植物病原青枯菌为例，青枯菌可以在土体和水体中长时间存活，欧洲有大量关于该病菌通过水渠系统传播污染的事件，给欧洲马铃薯、玫瑰等种植区带来巨大的威胁[48,49]．已有大量研究从污水或土壤中分离到侵染金黄色葡萄球菌[31,32]、铜绿假单胞菌[50,51]、鲍曼不动杆菌(*Acinetobacater baumannii*)[52]、洋葱伯克霍尔德菌(*Burkholderia cepacia*)[53]以及青枯菌[54]等病原细菌的噬菌体，这些噬菌体资源在抑制病原细菌的风险传播方面发挥重要作用．目前噬菌体疗法在农业种植业和养殖业中的研发应用受到较多的关注，在噬菌体资源的分离获取、宿主范围确定、分子表征、基因组学和蛋白组学的分析，噬菌体与靶细菌互作以及阻控病原细菌传播和感染的效果评估等方面均取得较大的突破．不过噬菌体疗法对一体化健康的生态意义以及作用机制仍需要大量系统研究．

2　噬菌体疗法的微生态机制

2.1　生态效应之病原细菌数量的控制

噬菌体对病原细菌数量削减是噬菌体疗法发挥作用的基础. Wang等人[7]发现，土壤中施入青枯菌的裂解性噬菌体能有效降低根际青枯菌的种群数量并降低植物的发病率. Fischer等人[55]的研究也表明，肉鸡口服空肠弯曲杆菌噬菌体能显著降低盲肠内容物中弯曲杆菌的数量，最高可降低2.8个数量级．虽然噬菌体很难完全清除环境中的靶细菌，但能将其控制在较低的水平，这对病原细菌数量依赖型的致病系统有重要意义．一些病原菌致病特征的表达取决于种群数量，即只有种群数量达到一定的阈值才启动致病系统、分泌毒力因子、引起病症. 以植物病原青枯菌为例，其毒力性状是由Phc(表型转换，phenotype conversion)调控的数量依赖型的系统控制[56]，其中3-OH棕榈酸甲基酯(3-OH PAME)是重要的信号分子[57]．青枯菌种群数量低时产生的3-OH PAME少，导致胞外多糖和外源酶合成减少，对植物的致病力大大降低[58]. 因此，噬菌体迅速侵染并裂解宿主细胞，降低宿主种群数量，一方面降低了病原细菌入侵宿主的可能性，另一方面也降低了病原细菌的致病能力(图2).

2.2　进化效应之抗性代价

与抗生素疗法相似，噬菌体疗法也不可避免地诱导靶细菌产生抗性．尤其是针对某一特定噬菌体，细菌很容易形成抗性机制，例如，侵染初期通过改变受体的空间结构[59]、产生胞外基质[60]或竞争排除抑制[61]等方式阻止噬菌体的吸附；通过限制性修饰系统(restrictive modification system, RM)[62]或CRISPR-Cas (clustered regularly interspaced short palindromic repeats (CRISPR)-associated protein)系统切割降解噬菌体的DNA[63]；宿主细菌为了减少噬菌体侵染种群中其他细菌，采取自我裂解的方式阻止噬菌体的有效增殖和释放[64]．不过，病原细菌进化获得噬菌体抗性是一个高成本的适应过程．噬菌体首先识别并作用于病原细菌表面的受体，如鞭毛[65]、菌毛[66]、荚膜多糖[67]等，继而开始侵染过程，这些受体可能同时与细菌的资源利用、生存竞争和致病性相关．当病原菌通过丢失噬菌体特异性受体获得抗性时，可能会减慢病原菌的生长[68,69]，降低病原菌对环境的适应能力[69]，损害错配修复基因[70]，降低致病相关基因的表达[71]等．Wang等人[7]研究发现，根际噬菌体胁迫下，进化后的病原青枯菌生长显著减缓，且青枯菌的噬菌体抗性与生长之间存在显著的负相关关系，表明噬菌体即使诱导高水平的抗性，青枯菌也不太可能恢复或达到很高的种群密度．此外，还发现青枯菌的噬菌体抗性与致病能力之间存在显著的负相关关系，即对噬菌体抗性越强的青

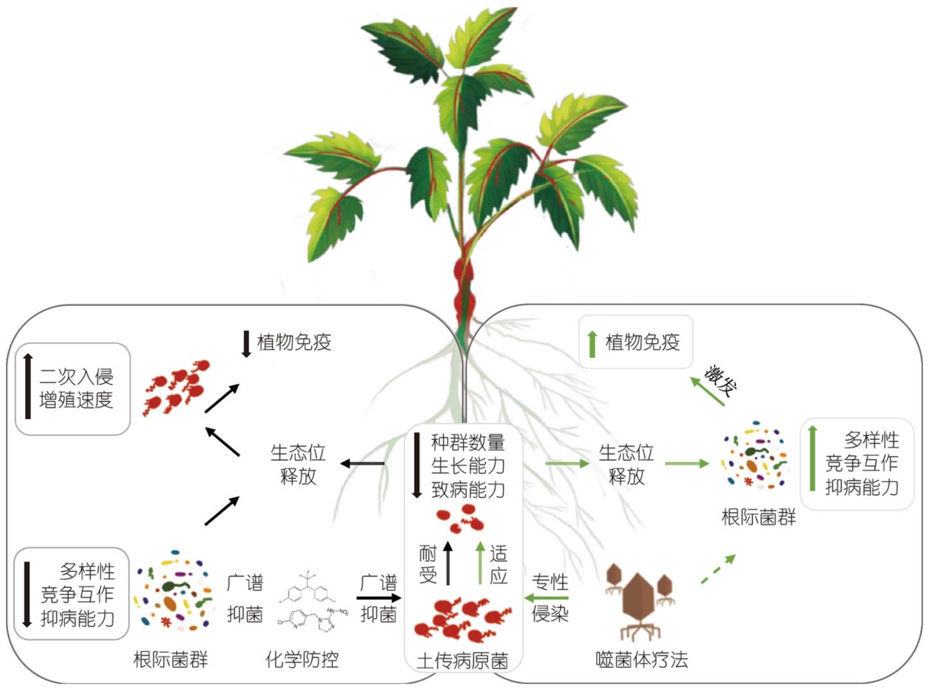

图 2　噬菌体疗法的微生态作用机制
Figure 2　Microecological mechanisms of phage therapy

枯菌，致病能力越弱[72]．所以噬菌体疗法的作用机制除了杀死细菌(敏感菌株)，还胁迫幸存者(抗性菌株)转化为生长和致病能力减弱的生态型，从而达到生态阻控的目标(图2).

2.3　噬菌体疗法之微生物群系调控

细菌和噬菌体之间的协同进化被认为是微生物群落中细菌多样化和变异的驱动因素．基于“杀死赢家(kill the winner)”动力学理论，噬菌体特异性压制环境中高丰度的病原细菌，有利于竞争者占据优势，增加群落的细菌多样性[73,74]．Morella等人[75]探究接种噬菌体对番茄叶际微生物群落的影响，发现噬菌体显著改变群落中优势物种的相对丰度，且影响叶际细菌群落的多样性．噬菌体侵染还可以通过改变环境中的营养资源和宿主细菌代谢来增加微生物群系多样性．Fazzino等人[76]研究发现，噬菌体裂解宿主释放的胞内物质可以作为营养物质，增加宿主菌互养共生细菌的生物量．Hsu等人[77]研究发现，噬菌体侵染通过影响宿主细菌的种群数量和代谢特性，进而对微生物种群产生级联影响．Wang等人[7]研究发现，病原细菌入侵显著改变微生物群落的结构，降低群落的多样性；而单独接种病原细菌的专一性噬菌体，对群落结构和多样性没有显著影响；同时接种病原细菌和噬菌体能一定程度恢复病原细菌入侵对微生物群落结构和多样性的扰动．说明病原菌专一性噬菌体对微生物群落多样性和结构的调控是一种间接作用：噬菌体有效降低病原细菌的数量，为土著微生物释放出更多的生态位(资源和空间)，使得群落中竞争能力强的物种获得种群增长的优势(图2)．接种噬菌体后，根际放线菌门(Actinobacteria)、变形菌门(Proteobacteria)和厚壁菌门(Firmicutes)中一些对病原青枯菌具有抑制作用的物种丰度显著富集[7]．Rasmussen等人[78]综述了噬菌体通过粪便移植改变了肠道微生物群系组成、宿主的代谢，进而改善宿主健康．

2.4　噬菌体疗法之真核宿主免疫

真核宿主(病原菌的宿主植物、动物、人体等)免疫系统能通过识别和清除作用抵御外来病原体的入侵．作为一种外源物，噬菌体在治疗动物病原菌感染时，不可避免要与真核宿主免疫系统相互作用．Roach等人[79]利用小鼠和计算机模拟多重耐药铜绿假单胞菌感染模型，结果发现噬菌体疗法在嗜中性粒细胞和

MyD88免疫激活的情况下才能表现出对病原细菌的清除效果. 基于噬菌体与嗜中性粒细胞之间的协同作用, 该研究提出了“免疫-噬菌体协同作用”的概念. 此外, 有研究还发现巨糖基转移酶编码基因的突变株在荚膜合成不足的情况下促进了肺炎克雷伯菌对噬菌体的抗性, 随后增强了巨噬细胞(免疫细胞)的吞噬作用, 表明该菌株更易被免疫细胞清除[80]. 说明真核宿主的免疫防御与噬菌体疗法可能存在协同作用, 共同抑制病原菌入侵, 维护病原菌宿主的健康. 虽然目前农业种植领域还未发现噬菌体与宿主免疫协同的现象, 但噬菌体对根际菌群的调控可能会进一步激发植物的免疫系统, 进而增强植物抵御病原菌入侵的能力(图2).

3　噬菌体疗法在一体化健康领域的挑战

3.1　病原菌种群、生理和基因特征的变异性

农业种养系统中可能同时存在金黄色葡萄球菌、粪肠球菌、大肠杆菌、肺炎克雷伯菌、铜绿假单胞菌和鲍曼不动杆菌等多种病原细菌[51,81]. 即使是一种病原细菌, 也可能在环境中呈现高度的多态性. 以青枯菌为例, 该病原细菌可分为5个生理小种、6个生化小种、4个基因型和大量序列变种[82]. 本实验室前期从全国不同地区、不同寄主植物中分离收集的1000多株青枯菌在基因型和生态型上呈现多样性, 即使来自同一植物的多株青枯菌也在生长和致病等能力上存在较大的变异性, 说明青枯菌在不同寄主植物-土壤系统中长期适应演化出丰富的基因型, 并表现出生理表型的多态性[72]. 噬菌体侵染的高度特异性, 不仅表现在物种水平、病原菌的生物膜和胞外多糖等生理特性改变, 也会影响噬菌体吸附和裂解效率[83,84], 所以, 某些专性噬菌体可能只感染一种基因型或生理型的病原细菌. 研究表明, 成熟的生物膜会延迟噬菌体的侵染, 而且生物膜深层细胞的低代谢率也会降低噬菌体的繁殖[85]; 另外, 病原细菌分泌的胞外多糖能覆盖病原细菌细胞表面的识别基团, 阻止噬菌体的识别和吸附[86]. 除了这些天然的噬菌体侵染阻碍物, 细菌也进化出一系列的抗噬菌体系统, 如限制性修饰系统、限制性修饰相关的防御基因岛系统(defence island system associated with restriction-modification, DISARM)、CRISPR-Cas系统、逆转录酶系统及化学防御机制等[87,88]来阻止噬菌体的侵染. 因此, 应用单一噬菌体往往无法有效抑制环境中多样的病原细菌, 成功的噬菌体治疗需要快速获得同时针对不同病原细菌的噬菌体配方[89].

3.2　噬菌体与宿主病原菌互作博弈的局限性

噬菌体疗法发挥作用的前提是能与病原细菌精确匹配, 也就是特异性识别与感染宿主菌, 这种识别特异性甚至达到细菌“株”的水平. 噬菌体的局限性首先体现在侵染裂解谱范围上. Zhao等人[90]发现, 当土壤中有多种类型病原菌时, 宿主特异性噬菌体抑制病原细菌的能力显著低于宽宿主范围的噬菌体(多价噬菌体). 其次, 噬菌体侵染病原菌是数量依赖型的, 当环境中的宿主细菌数量足够多且生理上和遗传上适合噬菌体感染时, 才能有利于噬菌体侵染增殖[91]. 如果目标细菌密度太低, 噬菌体与病原细菌相遇的概率会大大下降, 噬菌体的裂解速率也会降低, 甚至会转为溶原状态[92]. 此外, 单一噬菌体疗法与抗生素类似, 其作用效果会因病原菌的抗性进化而削弱. 为解决这些局限性, 除了筛选裂解谱广的噬菌体, 还需要定向驯化出具有更强侵染能力的噬菌体[93], 或构建噬菌体鸡尾酒等策略提高噬菌体疗法的稳定性. 值得注意的是, 有研究发现噬菌体是环境中抗生素抗性基因(antibiotic resistance genes, ARGs)的储存库和潜在的传播载体[94,95], 建议避免使用编码溶原性、毒力因子或携带ARGs的噬菌体. 评估农业环境中噬菌体-宿主病原菌-ARGs之间的关系对噬菌体疗法的应用推广、规避其环境风险具有重要意义.

3.3　噬菌体生产和应用的复杂性

作为具有生物活性的生物制剂, 在实际生产和应用过程中噬菌体是否能保持高活性决定了其治疗效果. 噬菌体的生产和运输主要包括扩繁、离心、纯化、包装、递送等步骤, 不同工艺程序都会影响其稳定性. 例如, 长时间高速离心会导致噬菌体断尾、温度过高会加速噬菌体的失活、直接冻干易造成噬菌体的破裂等. 目前已开发出多种策略来提高噬菌体产品的稳定性, 如添加保护剂在噬菌体表面形成保护膜、维持渗透压平衡和降低冰点等来维持噬菌体的活性. 在此基础上利用喷雾干燥、冷冻干燥、乳液和聚合技术等技术[96]获得稳定性高的噬菌体产品. 在应用过程

中噬菌体产品的稳定性受到递送(给药)途径、剂量、时间和宿主免疫等多种因素的影响. 口服噬菌体对抑制鸡体内空肠弯曲杆菌的定殖有较好的治疗效果[97], 但口服噬菌体可能会被蛋白酶水解或在酸性胃环境中失活; 肌内注射不同浓度的噬菌体对患有大肠杆菌源性败血症的鸡和小牛具有显著的保护作用[98], 但也可能会诱导严重的免疫反应. 在土壤-植物、土壤-水环境系统中, 温度、光照、水分及土壤等多种因素也会影响噬菌体的应用效果. 一般可以通过调整噬菌体的给药剂量、单次或多次治疗、利用脂质体(磷脂)作为噬菌体的传递载体[97]、通过微胶囊化或与抗酸剂共同给药来应对酸性环境[99], 结合多种递送途径(口服、注射、喷施等)[100]来提高噬菌体疗法应用的稳定性.

4 噬菌体疗法在一体化健康领域的机遇

4.1 一体化健康的需求

1990年农业部提出发展绿色食品产业: 提高食品的质量安全, 促进消费者健康; 保护农业生态环境, 促进农业可持续发展. 2018年“生态文明”被写入宪法, 2019年国家卫生健康委提出《健康中国行动(2019—2030年)》. 党的十八大以来, 党中央国务院高度重视农业绿色发展, 习近平总书记多次强调, 绿水青山就是金山银山. 2020年国家自然科学基金委员会设立了“土壤生物复合污染过程与调控”重大项目, 旨在通过科学研究进一步恢复和提升土壤健康. 农业发展需要牢固树立新发展理念, 以农业供给侧结构性改革为主线, 以绿色发展为导向, 以体制改革和机制创新为动力, 走出一条产出高效、产品安全、资源节约、环境友好的农业现代化道路. 化肥、农药“减量增效”已取得初步成效, 一定程度减轻了环境污染、提高了农产品质量. 但农业种植、养殖中的病原细菌泛滥, 严重阻碍了农业的绿色发展和人类健康. 据估计, 人类已知的每10种传染病中至少6种源自动物, 即人畜共患病; 植源性病原菌也存在通过食物链向人体传播的风险(https://www.cdc.gov/onehealth/basics/zoonotic-diseases.html). 为了有效抑制病原细菌, 同时减少化学农药和抗生素使用, 急需噬菌体这样精准靶向病原微生物的绿色疗法来实现农业绿色转型. 目前噬菌体也被用于农业生产的多个环节, 如种植、养殖中病原细菌的预防和治疗. 噬菌体疗法在农业领域的应用推广是服务于生命共同体与一体化健康的重要举措.

4.2 噬菌体疗法的技术突破

尽管噬菌体疗法具有其不可替代的优势, 但其效果往往受限于宿主范围窄以及病原细菌易形成对噬菌体的抗性进化. 为了提升噬菌体疗法的效率, 需要建立多样化噬菌体库和快速筛选噬菌体鸡尾酒配方的系统, 不再依赖单纯的分离培养技术. 可以结合培养组学、实验进化学、机器学习等技术, 构建基因组预测噬菌体抗性的模型, 结合病原菌-噬菌体生态互作特征和生物信息模型选择能够侵染不同病原细菌或同一病原细菌的不同抗性突变体的噬菌体鸡尾酒配方, 规避噬菌体宿主范围狭窄和病原细菌抗性突变的问题. 随着合成生物学的发展和对噬菌体侵染机制了解的不断深入, 可以通过基因编辑定向改造噬菌体, 如改变/扩大噬菌体的宿主范围以及增强噬菌体的裂解效率[101], 降低噬菌体毒性和免疫原性, 提高给药后噬菌体存活周期等[102]. 为了解决噬菌体应用的安全性问题, 利用表达纯化噬菌体编码的裂解酶、内溶素、溶菌酶等物质来抑制病原菌, 可以避免直接使用噬菌体. Yang等人[103]发现, 噬菌体裂解酶的重组蛋白V12CBD具有降低金黄色葡萄球菌毒力与增强免疫清除的多重功能, 为研究治疗包括耐甲氧西林金黄色葡萄球菌在内的超级耐药细菌感染提供了新的思路.

4.3 多种技术协同

除了病原微生物, 农业环境系统中还存在大量的土著细菌、真菌、原生动物等, 形成多营养级互作的微生态, 在抵御病原细菌入侵和感染过程中发挥着重要作用. 噬菌体疗法虽然有一定优势, 但其作用机制相对单一, 如果能够与竞争型有益菌以及捕食型原生动物和线虫等有益生物结合, 可以增加病原菌的生存胁迫, 有效避免噬菌体疗法的局限性, 进一步提高阻控病原细菌的稳定性. 研究发现, 青枯菌的专性噬菌体与产抗生素类物质的有益菌*Bacillus amyloliquefaciens* T-5协同不仅能限制病原青枯菌的生存能力[104], 还可以增加根际细菌群落的多样性和关键物种的丰度[105], 进而显著提高防控番茄土传青枯病的效果. 噬菌体与抗生素联用也是应对耐药菌的常用措施. 研究表明, 亚致死浓度的抗生素(β-内酰胺和喹诺酮类抗生素)可以增强噬菌体的裂解量. 这可能是由于低剂量的

抗生素抑制细菌细胞分裂，导致更短的潜伏期和更大的噬菌体爆发量，从而使噬菌体能更快地破坏剩余的细菌细胞[11]．此外，Lu和Collins[106]研究发现，噬菌体片段插入宿主基因组后，可以通过调控宿主的代谢网络，阻止抗生素抗性相关蛋白的表达，从而将抗生素疗法的效果提高几个数量级．多技术联合能够通过不同抑菌策略的协同效应，提高噬菌体治疗的成功率[107]．

5　总结与展望

当前农业生态系统面临的病原细菌入侵以及滥用农药或者抗生素等导致的病原细菌耐药性风险传播问题日益严峻. 尽管噬菌体疗法还面临诸如技术发展, 政策管控等多重挑战，但在生命共同体和一体化健康的时代需求下，利用噬菌体疗法的靶向性和动态调控等特性，发挥其优化微生态的优势是解决农业系统病原细菌危害和风险传播的不二选择．未来在加强噬菌体资源库的构建和信息共享、噬菌体-病原细菌互作机制、噬菌体合成生物学等基础研究和技术开发的基础上, 仍需优化噬菌体鸡尾酒的构建, 加大噬菌体扩增纯化及其衍生产品的开发和应用，建立噬菌体制剂个性化治疗的标准流程和应用技术规范；在评估噬菌体诱导真核宿主免疫和传播ARGs风险的基础上，还应关注病原菌获得噬菌体抗性的潜在风险，避免出现抗多种噬菌体的“超级细菌”；规范噬菌体产品的监管策略，推动噬菌体疗法在农业、环境和健康等领域的可持续发展应用, 促进一体化健康.

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Phage therapy for One Health approach: current status, challenges and opportunities

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Globalization is accelerating the planetary-scale transmission of bacterial pathogens among humans, animals, plants and environmental reservoirs, causing significant threats to public health, food security, environment and economy. Global decline in the development and availability of effective antibiotics and the rapid rise of antimicrobial resistance has generated renewed interest in phage therapy: a century-old practice to control bacterial pathogens using their viral parasites. In this paper, we first review the application of bacteriophages in controlling bacterial pathogens in agriculture, aquaculture and highlight their natural role in terrestrial and aquatic ecosystems. We then summarize the mechanisms of action of phage therapy: (i) pathogen density control; (ii) evolutionary steering of pathogen virulence via selection for phage-resistant but slow-growing and avirulent mutants; (iii) indirect effects on the composition and functioning of the resident microbiomes; and (iv) phage synergies with host immune system. Thirdly, we discuss the current challenges of phage therapy: (i) the phage specificity and diversity of pathogenic bacteria, which makes it difficult to design broad-spectrum phage cocktails; (ii) the rapid evolution of phage resistance via receptor modifications and expression of several anti-phage systems; and (iii) stability and functioning of phages in temporally and spatially varying environments that could constrain the use of phages in medical, agricultural and biotechnical contexts. Finally, we highlight the emerging strategies to improve the reliability and success of phage therapy applications.

pathogenic bacteria, micro-ecology, microbiome, phage therapy, One Health

doi: 10.1360/SSV-2021-0402