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Review 综 述

荧光假单胞菌防治果蔬病害的研究进展

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摘要：病原微生物侵染引起的果蔬病害日趋严重，现阶段果蔬病害的防治措施主要依赖化学防治，但长期大量施用合成农药的弊端如化学残留、环境污染、抗药性病原菌株出现等日益凸显。近年来，生物防治由于其安全性及高效、经济、环保等优点，成为研究热点。荧光假单胞菌(*Pseudomonas fluorescens*)分布广泛，施用方便，许多菌株能有效抑制多种病原微生物，成为最具应用价值的一类生防菌和根际促生菌。本文综述了荧光假单胞菌控制果蔬病害的生防效果、主要作用机制(直接寄生作用、营养物质和空间位点竞争、次生抗性代谢物、诱导宿主系统抗性)以及菌剂混配、物理方法、化学处理、分子技术在提高荧光假单胞菌生防效力等方面的研究进展，为荧光假单胞菌在生物防治领域的进一步开发利用提供一定的基础资料。

关键词：生物防治，荧光假单胞菌，果蔬病害，作用机制

病原微生物侵染引起的病害和腐烂是造成果蔬损失的重要原因，也是长期以来困扰果蔬产业发展的瓶颈[1]。传统控制果蔬病害主要依赖于化学杀菌剂，如仲丁胺、噻苯咪唑、抑霉唑和环酰菌胺等，但长期大量施用合成杀菌剂可能导致化学残留、环境污染、病原菌耐药性增强等问题，甚至会降低食品的安全性，因此，研究高效、经济、安全、环保的防治措施意义重大[1–2]。生物防治是指利用微生物之间的拮抗作用，选用对寄主无害而对病原微生物有明显抑制作用的拮抗微生物或微生物代谢产物来抑制病原物的生长、发育和繁殖，从而达到防治病害的目的[1–3]。近几年，生物防治由于其无毒环保、安全有效等特点，已成为果蔬病害控制的研究和开发热点[1–3]。目前，已有上百种真菌、细菌、放线菌等拮抗微生物成功应用于果蔬病害的生物防治，并有多种商业化生防

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菌剂陆续上市[1−2]。荧光假单胞菌(*Pseudomonas fluorescens*)，广泛分布于植物根际土壤和果蔬表面，许多菌株能在有效抑制病原物的同时，促进果蔬的生长和增产，因而受到各国研究者的关注，是国内外研究最早、报道最多的一类生防菌和根际促生菌[2,4]。30 余年来，对 *P. fluorescens* 生物防治的研究主要集中在其根际促生作用和对植物病害的防治上[2,4]，近年来，一些学者用 *P. fluorescens* 成功防治了草莓[5]、苹果[6−8]、柑橘[9−10]和香蕉[11]等多种果蔬的侵染性病害，极大地扩展了 *P. fluorescens* 的应用范围。由此，本文对 *P. fluorescens* 的生防效果、主要作用机制以及提高其生防效力的有效措施等方面进行了综述，以期为 *P. fluorescens* 在生物防治中的进一步开发与应用提供借鉴。

1 荧光假单胞菌

荧光假单胞菌属薄壁菌门假单胞菌科假单胞菌属，在进化上属于 rRNA Ⅰ群荧光假单胞菌 DNA 同源组，是一种化能异养型的革兰氏阴性菌，透射电镜下观察其菌体呈杆状，菌体形态呈单个或成对排列，具有极生鞭毛，运动能力强，与该属其他种类的典型区别在于能产生水溶性的黄绿色素，即青脓素，在 366 nm 紫外光照射下呈黄绿色或蓝白色荧光[2,4]。由于具有分布广、适应能力强、营养需求简单、繁殖快、定殖能力强、易于人工培养、遗传背景清楚、对人和环境无害、能防治多种病原物等特点，*P. fluorescens* 越来越受到专家学者的关注，成为近几十年来研究报道最多、最具应用价值的一类生防菌和根际促生菌[2,4,12]。

2 荧光假单胞菌的生防效果

大多数水果的 pH 较低，主要受病原真菌的侵染，而蔬菜则受到病原真菌和病原细菌的双重危害，但以真菌为主[1]。由于病原真菌一般通过孢子萌芽、形成腐生菌丝等方式侵染植物组织[1−2]，因而在评估某种微生物能否作为生防菌株时，首先需要确定该菌株抑制病原菌孢子萌芽和菌丝生长的能力[2,8]。研究人员通常采用离体试验(*in vitro*)和活体试验(*in vivo*)确定 *P. fluorescens* 的抑菌活性。研究表明，*P. fluorescens* 能有效抑制多种病原真菌孢子的萌发、游动孢子囊的产生以及病原菌的菌丝生长[2]，如 *P. fluorescens* 1-112、2-28、4-6 同病原菌在苹果汁液体培养基中共培养时，14 h 内，几乎完全抑制了扩展青霉(*Penicillium expansum*)、梨状毛霉(*Mucor piriformis*)和灰葡萄孢(*Botrytis cinerea*)的孢子萌发[8,13−14] (表 1)。

部分菌株的离体抑菌活性与活体试验的防治效果不一定呈正相关，Ghazanfar 等[15]报道，从番茄根际筛得的 1 株 *P. fluorescens*，在培养基平板上对念珠地丝菌(*Geotrichum candidum*)、粉红单端孢 (*Trichothecium roseum*) 、稻根霉菌 (*Rhizopus oryzae*)的菌丝生长都具有显著的抑制作用，但在番茄果实上接种后，仅能抑制酸腐病和软腐病的发生，对粉红霉病几乎没有抑制作用。因而，研究人员常常联用离体试验和活体试验，评估拮抗菌的生防效力。Ilhan 和 Karabulut[5]研究发现，*P. fluorescens* 122 能有效抑制 *B. cinerea* 的菌丝扩展，不论是采前施用还是采后施用，都能有效控制草莓灰霉病，防治效果几乎和化学杀菌剂环酰菌胺相当；Olanya 等[16−17]报道 *P. fluorescens* 2-79 能竞争抑制沙门氏菌(*Salmonella enterica*)，且使用 *P. fluorescens* 进行生物防治能有效降低工业加工成本。

此外，拮抗菌株的生防效力还与宿主品种有关，*P. fluorescens* 1-112、2-28、4-6 都能有效降低采后嘎啦、麦金托什、斯巴达苹果霉腐病的发病率和病斑

表 1. 荧光假单胞菌对不同病原菌的生防效果

Table 1. The biocontrol efficacy of *Pseudomonas fluorescens* against different kinds of pathogens

No.	Sources	Pathogen	Host	Biocontrol effect	Reference
122	Pea plants	*Botrytis cinerea*	Strawberry	*P. fluorescens* 122 was effective for the biocontrol of *B. cinerea* infection with pre- or post-harvest treatment, almost the same as commercial chemical fungicide.	[5]
1-112, 2-28, 4-6	The rhizosphere soil of pulse crops	*Penicillium expansum*, *Mucor piriformis*, *Botrytis cinerea*	Apple	*P. fluorescens* 1-112, 2-28 and 4-6 were highly effective for the inhibition of conidial germination of pathogens (over 90%), while the effect differed with apple varieties and pathogen types.	[8, 13–14]
–	Tomato growing fields	*Geotrichum candidum*, *Trichothecium roseum*, *Rhizopus oryzae*	Tomato	Dual culture assay revealed that *P. fluorescens* inhibited the radial growth of *G. candidum*, *T. roseum* and *R. oryzae*. The results *in vivo* showed that *P. fluorescens* provided good control (78.1%) of *G. candidum* and (82.2%) *R. oryzae*, but not to *T. roseum*.	[15]
2-79	Academic exchange	*Salmonella enterica*	Tomato	*P. fluorescens* 2-79 reduced risk of foodborne diseases caused by *S. enterica* via competitive inhibition.	[16]
2P24	Laboratory preservation	*Rhizoctonia solani*	Cotton	*P. fluorescens* 2P24 strongly inhibited the growth of *R. solani* when cultured with glucose, whereas not with fructose or mannitol culture.	[18]

–: indicates the strains were not numbered. The same below.

直径，菌株 1-112 和 2-28 对麦金托什苹果的生防效果甚至超过了商业生防菌剂 BioSave®和杀菌剂 Scholar®，但 3 株菌对美味苹果霉腐病几乎没有抑制作用[13]。此前，Wallace 等[8]用 *P. fluorescens* 防治苹果青霉病时，也发现这 3 株菌在麦金托什和斯巴达苹果上对 *P. expansum* 的抑制效果并不一致，他认为这可能和苹果不同品种间的营养成分差异有关。张燕等[18]的研究结果支持这一观点，他们发现，以葡萄糖为碳源时，*P. fluorescens* 2P24 的抑菌活性最强，蔗糖次之，而以果糖、甘露醇、葡萄糖酸钠、琥珀酸钠、马铃薯浸提液为碳源时，菌株 2P24 对立枯丝核菌(*Rhizoctonia solani*)没有抑制作用。

3 荧光假单胞菌防治果蔬采后病害的主要作用机制

了解拮抗微生物的作用机制，深入探究和掌握拮抗菌、病原物和宿主之间的互作效应，有助于提高拮抗菌的生防效力和提高靶标菌株的筛选效率，对扩展微生物源杀菌剂途径也具有重要意义[1,19]。目前国内外学者普遍认为，拮抗微生物的防治机理主要有以下四种作用模式：直接寄生作用、营养和空间竞争作用、产生抑菌物质以及诱导宿主抗性[19–20]。拮抗菌对病原微生物的作用机制可以以一种为主，也可以同时依赖多种机制，不同环境、不同宿主、不同病原物，其作用机制又可能表现不同[4] (表 2)。

3.1 直接寄生作用

P. fluorescens 能在病原菌菌丝上附着定殖，形成对病原菌的直接寄生作用，有的还能分泌一种或多种胞外水解酶，如几丁质酶(Chitinase，CHI)、β-1,3-葡聚糖酶(β-1,3-glucanase，GLU)、纤维素酶和蛋白酶等，裂解病原菌细胞壁或菌丝体，并能进一步抑制病原菌孢子发芽和芽管伸长[2,19]。

表 2. 荧光假单胞菌生物防治的作用机制

Table 2. Mechanisms of *Pseudomonas fluorescens* on biocontrol

No.	Sources	Pathogen	Host	Control methods	Mechanism	Reference
1-112, 2-28, 4-6	The rhizosphere soil of pulse crops	*Penicillium expansum*, *Mucor piriformis*, *Botrytis cinerea*	Apple	All three isolates produced protease, siderophores and VOCs, and could colonize the wounds of apples. In addition, isolate 2-28 was positive for the HCN biosynthesis gene, and both isolate 1-112 and 4-6 were positive for the gene encoding the production of PCA.	P+C+M	[8, 13–14]
P-72-10	The rhizosphere soil of tobacco	*Phytophthora nicotianae*	Tobacco	*P. fluorescens* P-72-10 produced protease, cellulose, siderophores and VOCs. Also, it effectively reduced MDA content and increased POD, PPO, PAL, CHI and GLU activity in tobacco seedlings.	P+C+M+I	[21–22]
PEF-5#18	Laboratory preservation	*Fusarium oxysopoyum*	Tomato	*P. fluorescens* PEF-5#18 could colonize in rhizosphere soil and extend inside tomato root-stem.	C	[23]
–	Academic exchange	*Penicillium italicum*	Citrus	*P. fluorescens* could inhibit spore germination, germ tube elongation and mycelial expansion of *P. italicum*, and rapidly grow in the wound of fruits, thus improving the CHI and GLU activities of citrus fruits.	C+I	[24]
FP7	Academic exchange	*Colletotrichum musae*	Banana	*P. fluorescens* FP7 was positive for the production of siderophores and DAPG.	C+M	[11]
ATCC 13525	Academic exchange	/	Tomato	*P. fluorescens* ATCC 13525 produced siderophores, and the content of iron in seeds soaked in bacterial fluid increased significantly.	C	[25]
FP7	Academic exchange	*Pythium aphanidermatum*	Turmeric plants	*P. fluorescens* FP7 was positive for the biosynthesis gene of PCA, DAPG, Plt, Prn and HCN. In addition, the activities of defense enzymes such as POD, PPO, PAL and SOD were enhanced by a combination of rhizome dip and soil drench of FP7 liquid formulation treatment.	M+I	[26]
LBUM223	The rhizosphere soil of potato	*Streptomyces scabies*	Potato	The isogenic mutant of LBUM223 (phzC$^-$), not producing PCA, was incapable to reduce *S. scabies* growth. PCA produced by *P. fluorescens* LBUM223 reduced *S. scabies* thaxtomin A production, leading to reduced virulence.	M	[27]
SF4c	The rhizosphere soil of wheat	*Xanthomonas*	Tomato	The tailocins produced by *P. fluorescens* SF4c caused damage to the cell envelope of strain *Xanthomonas*, resulting in a rapid leakage of intracellular materials.	M	[28]
ALEB7B	*Atractylodes lancea*	/	*Atracty-lodes lancea*	The VOCs produced by *P. fluorescens* ALEB7B could promote the growth and volatile oil accumulation of *Atractylodes lancea*.	M	[29]

(待续)

(续表 2)

WR-1	Laboratory preservation	*Ralstonia solanacearum*	Tomato	The VOCs produced by *P. fluorescens* WR-1 significantly inhibited the virulence of *R. solanacearum* via affecting protein metabolism.	M	[30]
UM16, UM240, UM256, UM270	The rhizosphere soil of *Medicago* spp. plant	*Botrytis cinerea*	Medicago truncatula	The VOCs produced by all *P. fluorescens* strains showed a high degree of antagonism against *B. cinerea* during confrontation assays, and significantly increased *Medicago truncatula* biomass and chlorophyll content.	C+M	[31]
SS101	Laboratory preservation	/	Tobacco	Eleven different compounds were detected in VOCs from *P. fluorescens*, and the VOCs could promote the growth of tobacco.	M	[32]
G20-18	Laboratory preservation	*Pseudomonas syringae*	Arabidopsis	*P. fluorescens* G20-18 could produce cytokinins and promote plant growth.	M	[33]
Sneb825	The rhizosphere soil of tomato	*Meloidogyne incognita*	Tomato	H_2O_2 biosynthesis related gene *RBOH1*, POD gene *Ep5C* expression and lignin biosynthesis related genes *Tpx1* expression of the samples treated by *P. fluorescens* Sneb825 reached the maximum level.	I	[34]
OCK	The rhizosphere soil of pea	*Erysiphe pisi*	Pea	*P. fluorescens* OKC could stimulate transcript accumulations of the Gα1 and Gα2 subunits of the heterotrimeric G protein, POD activities and phenol content in pea during the infection by *E. pisi*.	I	[35]
N21.4	The rhizosphere soil of tobacco	/	Blackberry	*P. fluorescens* N21.4 treatment caused increased expression of some flavonoid biosynthetic genes in blackberry fruits.	I	[36]

P: indicates parasitism and mycoparasitism; C: indicates competition for nutrients and space sites; M: indicates production of secondary resistance metabolites; I: indicates initiation of systemic resistance; /: indicates the pathogens were not clearly shown. The same below.

研究表明，拮抗菌寄生病原菌一般经过拮抗菌和病原菌彼此接触、相互识别、拮抗菌分泌水解酶并在寄主病原菌上生长繁殖等 4 个步骤[37]。*P. fluorescens* 1-112、2-28、4-6 均能在 *M. piriformis*、*B. cinerea* 和 *P. expansum* 菌丝上定殖寄生[8,13–14]，菌株 1-112 甚至能侵袭 *M. piriformis* 和 *P. expansum* 的孢子[8,13]。董国菊等[21]用 *P. fluorescens* P-72-10 的菌悬液处理烟草疫霉(*Phytophthora nicotianae*)菌丝，发现 *P. nicotianae* 菌丝出现分支增多、顶端膨大畸形、原生质渗漏等异常情况。

3.2 营养与空间竞争作用

与病原物竞争营养物质与空间位点是 *P. fluorescens* 最主要的作用机制之一。*P. fluorescens* 能在短时间内有效利用根际和果蔬伤口处有限的营养物质，迅速增长繁殖，抢占生态位点，使病原物得不到足够的营养物质和生存空间，无法进行孢子的萌发和生长繁殖等生命活动，从而抑制病害的发生[2,12]。现阶段普遍认为，拮抗菌能否在植物根际和果实伤口处成功定殖，快速繁殖形成数量优势菌是决定其能否发挥营养竞争和拮抗效力的重要因素[1–2]。张亮等[23]报道，*P. fluorescens* PEF-5#18 能良好定殖于番茄根际土壤并能扩展进入植物根茎内部，番茄根系表面、根内木质部、皮层细胞、细胞间隙、茎内维管均能观察到大量

P. fluorescens 供试菌株；笔者实验室研究也发现，不论是低温还是室温环境下，*P. fluorescens* ZX 都能在果实伤口处快速生长繁殖[24,38]。有些 *P. fluorescens* 甚至能在根际和果蔬表面形成生物膜(Biofilm, BF)，阻碍病原物接触、利用营养物质[2]。BF 是由细菌或真菌附着在生物或非生物体表面，被自身分泌的胞外聚合物(Extracellular polymeric substances，EPS)所包裹，形成具有一定结构和功能的细胞群体[39]。BF 能通过减低宿主免疫应答反应、抑制吞噬细胞等方式发挥对膜内微生物的保护作用，极大增强了膜内微生物对周围环境变化的耐受能力[39–40]。Wallace 等[14]报道，*P. fluorescens* 1-112、4-6 和 2-28 能在苹果伤口处形成致密的生物膜，但 3 株菌的最佳成膜时间在不同的培养基中不一样；随后，Allen 等[40]也发现，*P. fluorescens* PCL 1701 在高浓度营养下形成的 BF，其 EPS 含量较低浓度下的形成的 BF 高 3 倍，但杨氏模量较小，弹性高，粘性小，结构不稳定，而低浓度营养下形成的 BF 较其他处理明显更稳定。

Lugtenberg 等[3]认为，拮抗菌和病原物之间的竞争可分为资源竞争和干扰竞争。如上所述，拮抗菌通过特定的转运通道或高效的代谢水平，迅速消耗营养，增长繁殖，甚至形成 BF，都属于资源竞争。干扰竞争，又名植化相克，指拮抗菌能分泌出一种或多种生化物质，进而影响病原微生物的发芽、生长、生存和繁殖。不论是植物根际还是果蔬伤口处，铁元素的含量都非常低[2]。微生物一般需要 10^{-7}–10^{-6} 摩尔的生物可利用铁才能正常存活，在长期的进化中，很多 *P. fluorescens* 在低铁环境中，能分泌嗜铁素(Siderophore)。嗜铁素是一种小分子量(1–2 kD)的铁载体，可以高活性特异地螯合 Fe^{3+}离子，并作为一种运输工具将 Fe^{3+} 运入微生物细胞内[11]，*P. fluorescens* 可以通过这种方式和病原物竞争根际或果蔬伤口处有限的铁元素[2,11]。Nagata[25]研究发现，西红柿种子经 *P. fluorescens* 菌液浸泡后，植株中的铁元素含量显著增高。在低铁环境中，细菌和真菌都能产生嗜铁素，但细菌产生的嗜铁素和铁的结合作用更强，有些细菌甚至能进一步产生异源铁载体，夺取真菌嗜铁素中的铁离子[2,41]。如 *P. fluorescens* M114 产生的嗜铁素，与铁结合的稳定常数为 10^{32}，远高于某些病原菌如枯萎病菌的铁载体与铁结合的稳定常数 10^{29} [41]。

3.3 次生抗性代谢物

很多 *P. fluorescens* 能产生多种具有抗性的次生代谢物，如抗生素、细菌素、挥发性抑菌物质、毒蛋白、生物表面活性剂等，保护植物组织免受病原物侵染。

3.3.1 抗生素：分泌抗生素来抑制病原物的生长繁殖，这是 *P. fluorescens* 最早被确定的拮抗机理，也是其生物防治的热点。同一生防菌株可以产生多种抗生素，而不同的生防菌株也可产生相同的抗生素。抗生素是一类异源小分子化合物，低浓度的抗生素就可以阻滞病原物的生长[22]。1986 年，Gutterson 等[42]首次报道了 *P. fluorescens* 抗生素基因的克隆。后续研究表明，*P. fluorescens* 可产 2,4-二乙酰基间苯三酚(2,4-diacetylphloroglucinol，DAPG)[11,22,26]、酚嗪-1-羧酸(phenazin-e-1-carboxylicacid，PCA)[8,26–27]、藤黄绿菌素(pyoluteorin，Plt)[26,43]、吡咯菌素(pyrrolnitrin，Prn)[26]等多种抗生素。DAPG 和 Plt 为广谱抗生素，对多种病原物有抑制活性[22,26]；PCA 为吩嗪类物质，在细胞内作为电子载体传递电子到靶细胞，增加胞内超氧化物自由基，使靶

细胞中毒死亡[22,26–27]；Prn 在低浓度时破坏氧化磷酸化的偶联机制，在高浓度时阻止黄素蛋白和细胞色素 C 的电子运输[22,26]。Peeran 等[11]成功将能产 DAPG 的 *P. fluorescens* FP7 用于防治香蕉炭疽病；Arseneault 等[27]报道，*P. fluorescens* LBUM223 防控马铃薯赤霉病就是依赖分泌 PCA 实现的，且疥链霉菌(*Streptomyces scabies*)能刺激拮抗菌 *PHZC* 基因转录水平的提高；Wallace 等[8]采用聚合酶链式反应(Polymerase chain reaction，PCR)技术，确定 *P. fluorescens* 1-112 和 4-6 具有 PCA 的合成基因；随后，Prabhukarthikeyan 等[26]发现 *P. fluorescens* FP7 同时具有 PCA、DAPG、Plt、Prn、氢化氰(Hydrogen cyanide，HCN)的合成基因；而 Fernandez 等[28]从小麦根际分离到的 *P. fluorescens* SF4c 产生的一种新的抗生素——泰乐菌素，能粘附在黄单胞菌(*Xanthomonas*)细胞膜上，形成空隙损伤，引起内容物快速泄露，致使 *Xanthomonas* 裂解死亡。

抗生素的作用机制尚未完全清楚，Selin 等[44]的研究结果表明，PCA 并不是绿针假单胞菌 PA 23 生防核盘菌(*Sclerotinia sclerotiorum*)的主要机制，而是对其 BF 的形成起到至关重要的作用。此外，抗生素的使用会引发公众对食品安全、病原菌抗性等问题的担忧，因而在筛选拮抗菌，尤其是在筛选应用于防治采后果蔬病害的拮抗微生物时，应重点关注和推广使用不具备产抗生素能力的拮抗微生物[19]。

3.3.2 挥发性抑菌物质：生物防治是最具潜力和前景的新兴技术之一，但拮抗微生物缺乏治疗效果，即如果在拮抗菌到达作用点之前，病原菌已经感染，那么拮抗菌的生防效力就会降低甚至不起作用[45]。可喜的是，最新研究表明，一些拮抗菌在生长过程中能产生抑菌效果极强的挥发性物质(Volatile organic compounds，VOCs)，能协同作用抑制或杀死病原物，具有治疗效果，部分 VOCs 还能促进植物生长[29,45]。如 *P. fluorescens* 1-112、2-28、4-6 产生的 VOCs 能完全抑制 *P. expansum* 孢子的萌发和菌丝生长[8]；Raza 等[30]运用蛋白组学技术分析了 *P. fluorescens* WR-1 产生的 VOCs 抑制青枯菌(*Ralstonia solanacearum*)生长繁殖的可能机理；*P. fluorescens* UM16、UM240、UM256、UM270 均能产生 VOCs，主要的抑菌(*B. cinerea*)成分可能是含硫化合物如甲硫醇、二甲基硫醚、二甲基二硫醚、二甲基三硫醚等[31]；相似的，Park 等[32]报道，*P. fluorescens* SS101 能产生 11 种 VOCs，包括 1-十四碳烯-1-醇、2-丁酮和 2-甲基-N-1-十三碳烯等；而 Zhou 等[29]的研究结果表明，*P. fluorescens* ALEB7B 产生的几种含氮化合物，如甲酰胺和 N,N-二甲基甲酰胺、苯甲醛等，能有效促进苍术生长和挥发油的积累。由于利用拮抗菌 VOCs 进行的生物熏蒸防治病害，可适用于大多数产品的不同贮存和运输阶段，也可用于那些因太脆弱而无法进行液态杀菌剂处理的产品如草莓和葡萄，且拮抗菌与产品不直接接触，在一些风味测试中，经 VOCs 熏蒸的果实，风味不变，未在任何测试的水果表面检测到 VOCs 的残留，完全不会引起消费者对食品安全性的担忧，因而具有广泛的研究和应用前景[45]。

3.3.3 其他次生抗性代谢物：除了抗生素和 VOCs，*P. fluorescens* 还能产生其他多种次生代谢物，也能有效促进植物生长和抑制病原物。Grobkinsky 等[33]报道，丧失细胞分裂素合成基因的 *P. fluorescens* G20-18 对丁香假单胞菌(*Pseudomonas*

syringae)的生防效果显著降低，表明该菌株产生的细胞分裂素除了能促进植物生长发育以外，还能增强其生防效力。此外，HCN[26]、胞外多羟基丁酸聚合酶[46]、环脂肽[44]等也是 *P. fluorescens* 生物防治的热点。

3.4 诱导系统抗性

1983 年，Scheffer[47]发现 *P. fluorescens* 对榆长喙壳菌(*Ophiostoma ulmi*)毫无抑制作用，却能高效防控榆科植物叶斑病，由此认为 *P. fluorescens* 能激活宿主的自身免疫系统，进而对病原菌产生了抗性。现在一般认为，*P. fluorescens* 对宿主的诱导作用可能产生以下两个方面的效果[2]：(1) 提高宿主氧化应激能力，诱导宿主相关防御酶基因的上调表达，提高防御酶的活性。如 Prabhukarthikeyan 等[26]报道，使用 *P. fluorescens* FP7 菌液浸泡姜黄根茎、淋洗土壤，显著提高了姜黄过氧化物酶(Peroxidase，POD)、多酚氧化酶(Polyphenol oxidase，PPO)、苯丙氨酸解氨酶(Phenylalanine ammonia lyase，PAL)、过氧化氢酶(Catalase，CAT)、超氧化物歧化酶(Superoxide dismutase，SOD)等防御酶活性，有效抑制了根茎腐烂病并促进了姜黄生长和增产。(2) 影响宿主的次生代谢通路，调节通路中相关合成基因的表达，产生大量抗病性次生代谢物质，如植保素和酚类化合物等。Zhao 等[48]通过裂根法证明了 *P. fluorescens* Sneb825 可以诱导番茄产生系统抗性；尤杨等[34]进一步研究发现，经 *P. fluorescens* Sneb825 处理的番茄根系，活性氧合成相关基因 *RBOH1*、过氧化物酶基因 *Ep5C* 和木质素合成相关基因 *Tpx1* 上调，活性氧和木质素含量也显著提高；Patel 等[35]报道 *P. fluorescens* OCK 能促进豌豆异三聚体 G 蛋白 Gα1 和 Gα2 亚基的转录积累，提高 POD 活性和酚类物质含量；在此之前，Garcia-Seco 等[36]研究发现，田间施用 *P. fluorescens* N21.4 能影响蓝莓苯丙烷类化合物和类黄酮生物合成途径，诱导黄酮类物质生物合成基因大量表达，进而有效提高了蓝莓的品质。

4 提高荧光假单胞菌生防效果的措施

由于 *P. fluorescens* 的拮抗作用具有专一性、特殊性且易受外界环境因素影响等缺点，单独使用时常常达不到杀菌剂的防治效果，因而通过各种措施提高拮抗菌的生防效力，以便于生防制剂的应用和推广，是当今研究的热点和重点(表 3)。

4.1 不同菌剂混配

拮抗菌的抑菌谱、作用机制各不相同，不同类型拮抗菌株的混合使用可有效增强拮抗菌控制病虫害的能力。Istiqomah 等[49]研究发现，*P. fluorescens* 和枯草芽孢杆菌(*Bacillus subtilis*)联用，防控胡萝卜软腐欧文氏菌(*Erwinia carotovora*)比单独使用效果更好，优于杀菌剂 Agrept；*P. fluorescens* pf80 能和多种生防菌剂兼容，经 pf80、鹰嘴豆中慢生根瘤菌(*Mesorhizobium ciceri*)、萎锈灵和 Pusa 5SD 联合处理的鹰嘴豆种子发芽率最高，枯萎病发病率最低[50]。菌剂混配要特别注意拮抗菌之间的亲和性，因为生防制剂可能相互干扰、相互抑制，导致混配菌剂反而造成生防效果的降低，如 *P. fluorescens* 可有效增加葫芦巴的叶面积、鲜重、干重，提高氮、磷、钾含量与水分利用效率，但和苜蓿中华根瘤菌(*Sinorhizobium meliloti*)联用时反而会降低葫芦巴的产量[51]。

4.2 拮抗菌结合物理杀菌技术

P. fluorescens 对植物组织上已经感染的病原

表 3. 提高荧光假单胞菌生防效果的措施

Table 3. Treatments for enhancing the biocontrol efficacy of *Pseudomonas fluorescens*

Combined Treatments		No.	Sources	Pathogen	Host	Control effect	Reference
Mixture of inoculants	*Bacillus subtilis*	FPUB	Academic exchange	*Erwinia carotovora*	Tomato	The combination of '10^9 CFU/mL *B. subtilis* and 10^5 CFU/mL *P. fluorescens*', or '10^7 CFU/mL *B. subtilis* and 10^5 CFU/mL *P. fluorescens*' showed to be more effective.	[49]
	Mesorhizobium ciceri	Pf80	Academic exchange	*Fusarium oxysporum*	Chickpea	The seeds treated with Pusa 5SD+*Pf*80+*M. ciceri*+Vitavax power provided the highest germination, grain yield and the lowest wilt incidence.	[50]
Physical technology	Gamma irradiation	–	The rhizosphere soil of apple tree	*Penicillium expansum*	Apple	Combination of *P. fluorescens* and gamma irradiation (at 200–400 Gy) could decrease softening during apple storage.	[7]
	Modified atmosphere	1–112, 2–28, 4–6	The rhizosphere soil of pulse crops	*Botrytis cinerea*	Apple	On average, the combination of three isolates of *P. fluorescens* and modified atmosphere reduced the size of the grey mold lesion by 38.9%.	[2]
Chemicals	Salicylic acid	KSA1	Citrus leaves	*Xanthomonas*	Mexican lime seedlings	The application of *P. fluorescens* in combination with salicylic acid significantly reduced lesion number per leaf (72%) and disease severity (84%).	[52]
	Sodium bicarbonate	4–6	The rhizosphere soil of pulse crops	*Mucor piriformis*	Apple	Isolate *P. fluorescens* 4-6 in combination with Sodium bicarbonate significantly reduced Mucor rot decay, comparable to BioSave® and Scholar®.	[2]
Genetic improvement	Ribosome engineering	Pf-5	Laboratory preservation	*Phytophthora*	Tobacco	The yield of Plt and DAPG increased in engineering strain by 2.5 and 1.4 fold, respectively.	[43]
	Gene recombinantion	HC1-07	Laboratory preservation	*Gaeumannomyces graminis*	Wheat	Recombinant strain could produce PCA and cyclic lipopeptide simultaneously, and the biocontrol effect on wheat take-all disease was significantly increased.	[53]
	Metagenomics and gene recombinantion	Pf36	The rhizosphere soil of banana tree	*Radopholus similis*	Banana	The recombinant strains p4MCS-pf36 and p4Tn5-pf36 could secrete insecticidal proteins efficiently, and the lethality rate of *R. similis* increased significantly.	[54]

菌一般没有致死作用，而物理杀菌技术只能杀死组织表面的病原微生物，因此两者结合使用，可以优势互补，提高拮抗菌的生防效力。Mostafavi 等[7]发现，*P. fluorescens* 抑制 *P. expansum* 的能力同低剂量 γ 射线(200–400 Gy)相近，和 γ 射线联用可有效保持苹果的水分含量，降低金冠苹果的腐烂率；Wallace 等[2]报道，*P. fluorescens* 1-112、2-28、4-6 结合气调对控制采后苹果的青霉病和灰霉病有不同程度的增效作用。

4.3 拮抗菌结合化学物质

将拮抗菌和外源化学药物如低浓度杀菌剂、无机盐类、生长调节剂等配合使用也是提高拮抗菌生防效果的有效途径。Al-Saleh 等[52]报道，*P. fluorescens* 和水杨酸结合使用，能诱导墨西哥酸橙幼苗 POD 和 CAT 活性上升，防控 *Xanthomonas* 效果更好；Wallace 等[2]研究发现，碳酸氢钠能不同程度地提高 *P. fluorescens* 4-6 对采后苹果青霉病和霉腐病的生防效力。

4.4 分子技术对拮抗菌进行基因改良

近年来，分子生物学技术的快速发展，使得利用基因工程技术改造拮抗菌成为一个新的发展方向。最典型的是将苏云金芽孢杆菌的毒素蛋白基因导入 *P. fluorescens*，使之能够产生毒素蛋白，进而抑制根结线虫卵的孵化和幼虫的发育[48]；相似地，Chen 等[54]将蛋白酶基因 *pase4* 导入 *P. fluorescens* pf36，成功构建出基因重组菌株 p4MCS-pf36 和 p4Tn5-pf36，显著提高了其抑制香蕉穿孔线虫(*Radopholus similis*)的能力；Xie 等[43]以 *P. fluorescens* Pf-5 为出发菌株，运用核糖体工程技术获得一株 Plt 产量提高 2.5 倍、DAPG 产量提高 1.4 倍的突变株 R55，抑菌活性大幅增强；Yang 等[53]将 *P. synxantha* 2-79 的 PCA 合成基因导入 *P. fluorescens* 中，使得重组菌 HC1-07PHZ 能同时产生 PCA 和环脂肽，增强了其对小麦全蚀病的防治效力。

5 结语和展望

经过多年的努力，生物防治的研究已经取得了大量成果。*P. fluorescens* 对多种病原物都具有很强的生防效力，也可与其他拮抗菌、物理杀菌技术、化学物质处理结合使用，分子生物学技术的广泛渗入使得 *P. fluorescens* 获得更诱人的生防效果。但在实际应用中，仍有诸多问题亟待解决，比较突出的有以下几个难题。(1) 生防机制不够清晰。由于拮抗菌、病原物和宿主都是活的有机体，三者相互接触后的致病及诱抗作用是一个极其复杂的动态变化过程，相互作用模式多种多样，三者互作中信号的启动和传递等机理均不明确，这也是生物防治的重点和难点[1,55]。(2) 易受环境影响，生防效果不稳定。低温、高温、缺水、洪涝、高盐、紫外辐照等生态环境中的各种胁迫都有可能干扰拮抗微生物的生长繁殖，进而影响其生防效力和防治效果。(3) 拮抗菌株及基因工程重组菌株的安全性。大多数拮抗菌株是从根际土壤或果蔬表面筛选得到，为果蔬自然存在的正常菌落，但拮抗菌株进入商业化生产之前必须经过严格的安全性评价。

因此，未来应加强以下几方面的研究：(1) 采用多种方法，尤其是运用分子生物学技术深入研究生防菌株的拮抗机制，进一步探讨和确定拮抗菌、病原物、宿主之间的互作模式；(2) 继续筛选抑菌谱广、生防效力高、抗逆性强的菌株，并寻求适宜的处理方法，提高生防菌对环境变化的耐受能力；(3) 食品、植保、生态、信息等方面背景

研究人员开展协同研究，联合使用多种防治病害技术，强化研究其相互协同的作用条件和机制，实现优势互补；(4) 严格分析拮抗菌株，尤其是基因改良菌的生态安全性，应用于采后果蔬病害的拮抗菌株还需进行系统的毒理学评估。

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Advances in research for controlling fruits and vegetables diseases by using *Pseudomonas fluorescens*

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Abstract: Fruit and vegetables diseases are always caused by pathogenic microorganisms and traditionally, chemical control such as fungicide treatment was widely applied, which helped to cause chemical residues, environmental pollution and resistance in fungi, etc. Alternatively, microbial biocontrol methods were favored due to their safety, effectiveness and environmental harmlessness. In this paper, we reviewed the update investigations about the biocontrol effect and involved mechanisms of *Pseudomonas fluorescens*, one kind of biocontrol bacteria and plant growth-promoting rhizobacteria. We provided the basic information of *P. fluorescens* on biological control and discussed related mechanisms including parasitism, competition for nutrients and space sites, production of secondary resistance metabolites and initiation of systemic resistance, responsible for the biocontrol effect. Also, we illustrated the current research result for the improvement of biocontrol efficacy, such as inoculant mixing, physical methods, chemical treatments and molecular technologies.

Keywords: biocontrol, *Pseudomonas fluorescens*, diseases of fruits and vegetables, mechanism

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