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Research Article 研究报告

一种自溶性产酶溶杆菌新菌株 LE16 对温室番茄灰霉病的抑制作用

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Li Suping, Yuan Ling, Chi Shaoyi, Sui Zongming, Huang Jianguo. Suppression of tomato gray mold by a new strain of autolytic *Lysobacter enzymogenes* (LE16) in greenhouse. *Acta Microbiologica Sinica*, 2022, 62(10): 3871–3885.

摘　要：【目的】我国人多地少，设施农业日益普及，温室栽培保障了番茄的有效供给，在温室中种植的番茄容易发生灰霉病，造成减产。生物防治产品对人畜安全，环境友好，但亟待增加生防微生物种类，提高防效。【方法】以自主分离的自溶性产酶溶杆菌(*Lysobacter enzymogenes*) LE16 为对象，利用纯培养试验明确其发酵液对灰霉病菌(*Botrytis cinerea*)的拮抗作用；生物化学、液相色谱-质谱联用和转录组技术从生防菌合成分泌的胞外水解酶、抗菌物质和病菌基因差异表达等方面，揭示生防菌拮抗病原真菌的机制；温室盆栽试验评估菌株 LE16 发酵液对番茄灰霉病的防治潜力。【结果】LE16 能合成分泌铁载体、多种与抗病性相关的酶类(包括磷酸酶、蛋白酶、纤维素酶、β-1,3-葡聚糖酶和溶菌酶)以及 9 种抗真菌物质(毒菌素 D、N-十一烷基苯磺酸、利福布汀、纳奈霉素、替加环素、米诺环素、间甲酚、肉桂酸和环戊酮)和激活植物系统获得性抗性的 P-水杨酸。LE16 发酵液粗提物显著抑制灰霉病菌生长繁殖，抑制率为 34.99%–100%，显著高于产酶溶杆菌 HYP18 和撕裂蜡孔菌 HG2011。此外，LE16 发酵液显著诱导灰霉病菌基因的差异表达，涉及蛋白质合成、DNA 复制与修复、信号转导等多个生物过程、细胞成分和分子功能，以及甘氨酸、丝氨酸、苏氨酸和赖氨酸等代谢通路。在温室试验中，LE16 发酵液显著增加了番茄叶片的抗氧化酶(超氧化物歧化酶和过氧化物酶)活性，降低了病菌对细胞膜的伤害作用，对灰霉病的抑制效果为 72.54%–74.42%，略低于化学农药嘧霉胺。【结论】LE16 能通过合成分泌胞外水解酶、铁载体、抗

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真菌活性物质，诱导植物产生抗病性等多种机制，防治温室番茄灰霉病，具有潜在的应用前景。

关键词：产酶溶杆菌；自溶细菌；灰霉病菌；液相色谱-质谱联用；转录组

Suppression of tomato gray mold by a new strain of autolytic *Lysobacter enzymogenes* (LE16) in greenhouse

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Abstract: [Objective] Facility agriculture has experienced a surge in populairty in China which features a large population and little arable land. Greenhouse cultivation guarantees tomato security in China. However, greenhouse tomato are prone to gray mold (induced by *Botrytis cinerea*), leading to huge yield loss. Biocontrol is safe for human being and animal and environmentally friendly. It is urgent to seek more biocontrol microorganisms to improve the control effect. **[Methods]** In the current series of experiments, the biocontrol potentitial of a self-isolated new strain (LE16) of autolytic *Lysobacter enzymogenes* was explored. To be specific, pure culture was carried out to test the antagonism of LE16 fermentation broth against *B. cinere*a. Biochemical analysis, liquid chromatography-mass spectrometry, and transcriptome techniques were used to clarify the synthesis and secretion of extracellular hydrolases and antifungal compounds of LE16, and differentially expressed genes in *B. cinerea*, with a view to elucidating the antifungal mechanism. The inhibitory effect of LE16 fermentation broth on tomato gray mold was evaluated with the pot experiment in a greenhouse. **[Results]** LE16 synthesized and released siderophore, a variety of extracellular enzymes related to disease resistance (including phosphatase, protease, cellulase, β-1,3-glucanase and lysozyme), 9 antifungal compounds (ustiloxin D, N-undecylbenzenesulfonic acid, rifabutin, nanaomycin, tigecycline, minocycline, m-cresol, cinnamic acid and cyclopentanone), and P-salicylic acid that is able to stimulate systemic acquired resistance in plants. The crude extract of LE16 fermentation broth significantly inhibited the growth and reproduction of *B. cinerea*, and the inhibition rate was 34.99%–100%, much higher than those of *L. lysobacterium* HYP18 and *Ceriporia lacerata* HG2011 on solid culture. Additionally, LE16 fermentation broth significantly induced the differential expression of genes in *B. cinerea*. The differentially expressed genes were involved in biological processes, cellular components, molecular functions (e.g., protein synthesis, DNA replication and repair, and signal transduction), and metabolic pathways related to proteins, glycine, serine and threonine. In the greenhouse experiment, LE16 fermentation broth significantly enhanced the activity of antioxidant enzymes, including superoxide dismutase and peroxidase, in tomato leaves, and alleviated the damage to cell membranes caused by the pathogen. The inhibitory effect against gray mold varied from 72.54% to 74.42%, slightly lower than that of the chemical pesticide pyrimethanil. **[Conclusion]** LE16 suppresses the incidence of tomato gray mold in the greenhouse by multiple mechanisms, such as the synthesis and release of extracellular hydrolases, siderophore and antifungal compounds, and the

induction of resistance to disease in the plants. Thus, LE16 shows a promising potential in controlling tomato gray mold in the greenhouse.

Keywords: *Lysobacter enzymogenes*; autolytic bacteria; *Botrytis cinerea*; liquid chromatography-mass spectrometry; transcriptome

我国人多地少，设施农业日益普及，温室栽培保障了番茄的有效供给[1–2]，在温室中种植的番茄容易发生灰霉病，造成减产。番茄灰霉病的致病菌为灰葡萄孢，属子囊菌门(*Ascomycota*)、核盘菌科(*Sclerotiniaceae*)、葡萄孢属(*Botryotinia*)[3–4]，感染葡萄、番茄和莴苣等 1 400 种植物的花、茎、叶、果实[5]，在温度适宜和高湿条件下引起灰霉病。番茄灰霉病全球发生，生育期内反复多次发病，造成减产甚至绝收，经济损失巨大[6–7]。目前，化学农药是防治番茄灰霉病的主要方法[8]，但长期、大量、频繁施用化学农药导致病原菌产生耐药性，使其防治效果降低或失效[9]。此外，环境和农产品中积累的化学农药对人畜健康造成风险[10]。生物防治对人畜安全、环境友好[11]，为番茄灰霉病的防治提供了新的选择。但现有的防治番茄灰霉病的微生物菌株不多，防治效果因环境不同而异，亟待增加防治菌株的种类，稳定和提高防治效果。

目前，用于防治番茄灰霉病的生防细菌主要有解淀粉芽孢杆菌(*Bacillus amyloliquefaciens*)、枯草芽孢杆菌(*B. subtilis*)、乳芽孢杆菌(*Lactobacillus* sp.)、假单胞菌(*Pseudomonas* sp.)等，它们均对灰葡萄孢有拮抗作用[10]，可有效抑杀番茄叶片和果实上的灰葡萄孢[12]，但目前少有溶杆菌防治灰霉病的报道。溶杆菌广泛存在于海洋、湖泊、江河、土壤、热泉等复杂多样的自然生境中，种类和株系繁多，具有生产抗生素和防治植物病害的潜力[13]，如产酶溶杆菌 N4-7[14]、C3[15]和 OH11[16]，抗生素溶杆菌 13-1[17]，胶状溶杆菌 OH17[18]，变棕溶杆菌 OH23 等[19]。产酶溶杆菌 C3 能防治由立枯丝核菌(*Rhizoctonia solani*)引起的草坪褐斑病和由禾谷镰刀菌(*Fusarium raminearum*)引起的小麦赤霉病[15]。产酶溶杆菌 OH11 能分泌蛋白酶、几丁质酶、β-1,3-葡聚糖酶、纤维素酶等多种胞外水解酶和热稳定抗真菌因子，防治多种植物的真菌病害[16]。

我们自主分离获得产酶溶杆菌新菌株 LE16，兼具促进植物生长和防治病害的作用，能活化土壤有机氮磷，改善植物营养[20]，有效防治烤烟白粉病、辣椒疫病和烤烟赤星病[21–22]。在液体培养过程中，LE16 发生自溶[21]，意味着该菌株能分泌多种酶类，既降解自身成分，也可能使植物病原真菌的某些成分发生降解，进而产生较强的抑菌作用。本研究的主要目的是：(1) 了解菌株 LE16 发酵液粗提物对灰葡萄孢的抑制效果；(2) 明确 LE16 发酵液对温室番茄灰霉病的防治作用；(3) 利用代谢组和转录组技术，结合生化分析，揭示菌株 LE16 拮抗灰葡萄孢的生化和分子机制。

1 材料与方法

1.1 材料

植物：西红柿种子(*Solanum lycopersicum* L. cv.中蔬四号)购于河北省青县兴运蔬菜良种繁育中心。

化学农药：80%嘧霉胺(有效成分含量为 80%)，山东百农思达生物科技有限公司。

菌株：产酶溶杆菌 LE16 (GenBank 登录号：MK044898；CGMCC 保藏编号：No. 14215)和

HYP18[23] (GenBank 登录号：MT377319)分别从云南省玉溪市植烟土壤(102°30′E，24°14′N)和重庆市北碚区缙云山竹林土壤(106°17′E，29°41′N)中分离获得；撕裂蜡孔菌 HG2011[24–26] (GenBank 登录号：MT675050；CGMCC 保藏编号：No. 14215)从重庆市缙云山马尾松林土壤(106°40′E，29°84′N)中分离获得；灰葡萄孢由西南大学植物生态与病理研究所提供。在试验前，分别将 LE16 和灰葡萄孢接种在牛肉膏蛋白胨琼脂培养基(nutrient agar，NA；液体培养基不加琼脂，NB)和马铃薯葡萄糖琼脂培养基(potato dextrose agar，PDA；液体培养基不加琼脂，PDB)上，28 °C 暗培养 3–5 d 活化备用(下同)。

生防菌发酵液及粗提物：取活化后的菌株，接种于 NA (LE16 和 HYP18)和 PDA (HG2011)上，28 °C 暗培养 3–7 d，无菌水洗涤培养基表面的菌体或孢子，并稀释至 1×10^4 cells/mL。分别配制 NB 和 PDB，置于发酵罐中(型号：BLB10-50SJ-UIP，上海佰伦生物科技有限公司生产)常规制备 LE16、HYP18 和 HG2011 发酵液(28 °C、150 r/min，7–14 d；LE16 发生自溶)。分别取上述发酵液，过滤后加入 100 mL 乙酸乙酯萃取，40 °C 旋转蒸干，获得 LE16、HYP18 和 HG2011 发酵液粗提物。

灰葡萄孢孢子悬液：取灰葡萄孢菌落边缘的菌饼(ϕ=5 mm)，接种于 PDA 上，暗培养 5 d，无菌水洗涤 PDA 表面的孢子，并稀释至 4.5×10^5 孢子/mL。

1.2 LE16 分泌水解酶和铁载体

将 LE16 分别接种在含卵磷脂、牛奶、刚果红、苯胺蓝、灭活的金黄色葡萄球菌悬液、CAS 的检测培养基上，暗培养 2–7 d，菌落周围出现透明的水解圈或特有的彩色圈表示 LE16 能产生磷酸酶、蛋白酶、纤维素酶、β-1,3-葡聚糖酶、溶菌酶和铁载体。

1.3 LE16 发酵液粗提物对灰葡萄孢的抑制作用

分别取 LE16、HYP18 和 HG2011 发酵液粗提物，溶解于 0.4%的甲醇溶液的发酵液粗提物母液。当灭菌后的 PDA 冷却至 45–50 °C 时，分别加入发酵液粗提物母液，使其浓度分别达到 0%、0.025%、0.050%、0.100%和 0.200% (空白对照加入相应浓度的甲醇溶液)。在 PDA 中央接种 ϕ=5 mm 的灰葡萄孢菌饼，重复 4 次，暗培养 5 d，测定病菌菌落面积，按公式(1)计算抑制率。此外，在粗提物浓度为 0%和 0.05%的处理中，取菌落边缘的灰葡萄孢菌丝，显微镜观察菌丝形态。

$$\text{抑菌率}(\%)=\frac{\text{对照组病原菌菌落面积}-\text{处理组病原菌菌落面积}}{\text{对照组病原菌菌落面积}}\times100 \quad (1)$$

1.4 LE16 发酵液中抗菌物质分析

取 5 mg LE16 发酵液粗提物溶解于 0.4%甲醇溶液中，5 °C 涡旋 30 s，40 kHz 超声粉碎 30 min，–20 °C 静置 30 min 沉淀蛋白，5 °C 13 000×*g* 离心 15 min，将上清液转移到样品瓶中进行液相色谱-质谱(liquid chromatograph-mass spectrometer，LC-MS)分析。色谱条件：20 μL 样液，ACQUITY BEH C_{18} 色谱柱(100 mm×2.1 mm；Waters，Milford)，流动相 A 为 95%水+5%乙腈，流动相 B 为 47.5%乙腈+47.5%异丙醇+5%水，流速 0.40 mL/min。质谱条件：电子冲击电离源，离子喷雾电压 5 500 V，毛细管温度 550 °C，扫描采集模式扫描范围 35–500 *m*/*z*，扫描间隔 0.3 s，喷雾气体、辅助气体、加热气体、气帘气体和碰撞气体均为氮气；质谱设置参数：0.5 sigma 窗口值，5 000 EI 谱界值，0.5 min 保留时间界值，0.5 Da *m*/*z* 界值，70% EI 相似度界值，70%鉴定评分界值。

1.5 LE16 发酵液粗提物对灰葡萄孢基因表达的影响

取 1.3 中的对照和 0.05%发酵液粗提物处理的病原菌菌丝，将其置于液氮中冷冻，送往上海美吉生物科技有限公司进行转录组分析。具体方法是：用 TRIzol®试剂(真菌 RNA 纯化试剂；根据制造商的说明书，Invitrogen 公司)提取菌丝中总 RNA，DNase I (TaKaRa)去除基因组 DNA。提取的 RNA 经纯化和定量后，用 TruSeq RNA Sample Preparation Kit (Illumina)构建互补 DNA (cDNA)文库，PCR 扩增，Novaseq 6 000 测序平台(Illumina)进行 cDNA 测序。

1.6 LEFL 对温室番茄灰霉病的抑制作用

防治番茄灰霉病的试验在重庆市西南大学自然光温室中进行(2020 年 5–7 月)。将 30 日龄的番茄幼苗种植于 5 L 培养盆中(每盆 1 株)，设置(1) 对照(CK)；(2) 只接种病菌(PI)；(3) 接种病菌前喷施发酵液(LEFL+PI)；(4) 接种病菌前喷施化学杀菌剂(CF+PI)；(5) 接种病菌后喷施 LEFL (PI+LEFL)；(6) 接种病菌后喷施化学杀菌剂(PI+CF)等处理。接种病菌的方法为整株喷施灰葡萄孢孢子悬浮液(4.5×10^5 孢子/mL)，以湿润叶片滴水为度。在 LEFL+PI 和 CF+PI 处理中，于病菌接种 36 h 前分别喷施 LE16 发酵液和 80%嘧霉胺(使用时稀释 1 800 倍)；在 PI+LEFL 和 PI+CF 处理中，发酵液和嘧霉胺则在接种病原菌 36 h 后喷施。在 CK 处理中，整株喷施无菌水。在发酵液和无菌水中均加入少许吐温 20。试验采用完全随机设计，每处理设 4 次重复(每重复 25 盆)。

实施试验处理后，每天观察并统计番茄植株发病情况，当 PI 处理中的植株发病率达到 40%–50%时，统计并记录番茄植株病情状况，病级数分为 0–5 级：0=没有感染或<1%；1=1%–25%；2=26%–50%；3=51%–75%；4=76%–90%；5>90% (以叶片感染面积计)。病情指数和防治效果按照公式(2)和(3)计算[27]。

$$\text{病情指数}=\frac{\text{病情级数}\times\text{相应的叶片数量}}{\text{最大病情级数}\times\text{总的叶片数量}}\times100 \quad (2)$$

$$\text{抑制效果}(\%)=\frac{\text{只接种病菌组病情指数}-\text{处理组病情指数}}{\text{只接种病菌组病情指数}}\times100 \quad (3)$$

1.7 LEFL 对番茄叶片抗氧化酶活性、膜透性和丙二醛含量的影响

上述 1.6 温室盆栽试验统计并记录番茄植株病情状况的同时，每个处理分别随机选择 4 个盆钵，采集植物叶片。分别以氮蓝四唑分光光度法和愈创木酚法测定番茄叶片超氧化物歧化酶(superoxide dismutase，SOD)和过氧化物酶(peroxidase，POD)活性[28]，以硫代巴比妥酸(thiobarbital，TBA)法测定丙二醛含量，并通过电解质渗透法测定叶片细胞膜透性[28–29]。

1.8 数据处理与统计分析

在美吉生物科技有限公司的云平台(https://cloud.majorbio.com)上，用 Progenesis QI 2.3 软件(Waters，Milford)进行 LC-MS 的数据处理，保留总相似度≥800，填充度≥0.5%，碎片存在度≥800 的匹配物质。

用 Trinity 软件拼接 cDNA 文库；BLASTX 软件检索 NCBI 数据库中的基因序列，基因本体(gene ontology，GO)和京都基因与基因组百科全书(Kyoto encyclopedia of genes and genomes，KEGG)数据库中转录本序列编码的蛋白质，并注释基因功能(临界值 $E<1.0\times10^{-5}$)；Goatools 和 KOBAS 进行 GO 功能富集和 KEGG 通路分析；DESeq2/DEGseq/EdgeR 软件进行差异基因表达分析。此外，分别随机选取 8 个显著上调和下调的差异基因，利用实时荧光定量聚合酶链式反应(real-time fluorescent quantitative

polymerase chain reaction, RT-qPCR)对其进行验证。

用 Excel 2017 进行数据基本计算，SPSS 23.0 进行单因素方差分析，用 Duncan's 检验法进行差异显著性多重比较($P \leqslant 0.05$)，用 Excel 2017、Origin 9.0 和 R3 绘制图表。

2 结果与分析

2.1 胞外酶和铁载体的产生

将 LE16 分别接种于含卵磷脂、牛奶、刚果红、苯胺蓝、灭活的金黄色葡萄球菌悬液、CAS 的检测培养基上，培养 2–7 d 后均可观察到菌落周围出现透明圈或颜色变化(图 1)，说明 LE16 能分泌磷酸酶、蛋白酶、纤维素酶、β-1,3-葡聚糖酶、溶菌酶和铁载体。

2.2 菌株 LE16 发酵液粗提物的抑菌活性

在 PDA 培养基上，LE16 发酵液粗提物的浓度从 0.025%提高到 0.200%时，对灰葡萄孢菌丝生长的抑制率从 34.99%提高到 100%，显著高于 HYP18 和 HG2011 (图 2)。镜检结果表明，当发酵液的浓度为 0.050%时，灰葡萄孢的菌丝发生肿胀、空泡化、畸形和断裂等现象(图 3)。

2.3 菌株 LE16 发酵液中的抗菌活性物质

LC-MS 检测表明，LE16 发酵液含有毒菌素 D、N-十一烷基苯磺酸、利福布汀、纳奈霉素、替加环素、米诺环素、间甲酚、肉桂酸和环戊酮等 9 种抗真菌物质，以及能激活植物系统获得性抗性的信号分子 P-水杨酸(表 1)。

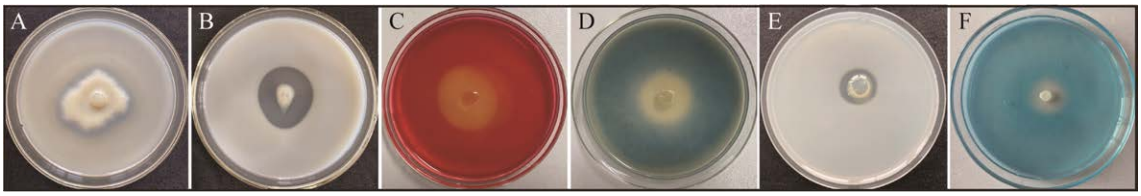

图 1 产酶溶杆菌 LE16 分泌胞外水解酶和铁载体

Figure 1 The production of extracellular hydrolases and siderophores by *L. enzymogenes* LE16. A: phosphatase; B: protease; C: cellulase; D: β-1,3-glucanase; E: lysozyme; F: siderophores.

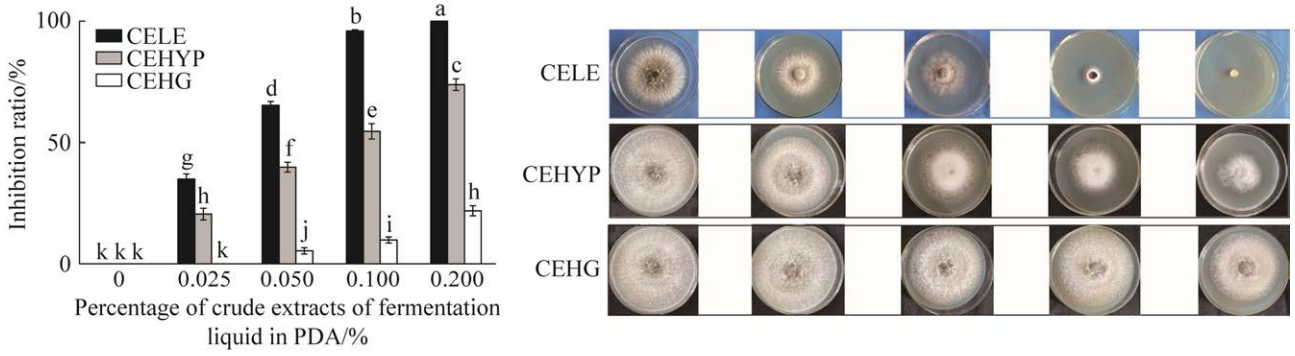

图 2 产酶溶杆菌 LE16 发酵液粗提物对灰葡萄孢的抑制效果

Figure 2 Inhibitory effects of crude extracts from *L. enzymogenes* LE16 fermentation liquid against *B. cinerea*. Values are means±standard deviation; different lowercase letters indicate significant differences at $P \leqslant 0.05$ (Duncan's multiple range test). CELE: crude extracts from LE16 fermentation liquid; CEHYP: crude extracts from HYP18 fermentation liquid; CEHG: crude extracts from HG2011 fermentation liquid.

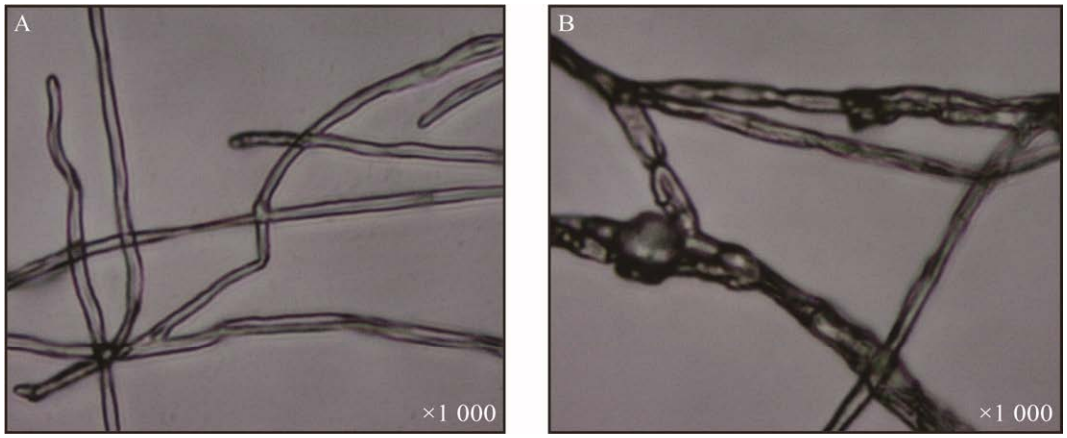

图 3　产酶溶杆菌 LE16 发酵液粗提物对灰霉病菌菌丝形态的影响

Figure 3　Changes in the mycelial morphology of *B. cinerea* caused by crude extracts from *L. enzymogenes* LE16 fermentation liquid. A: control (CK); B: crude extracts from LE16 fermentation liquid.

表 1　产酶溶杆菌 LE16 发酵液中的抗菌物质

Table 1　Antifungal substances present in *L. enzymogenes* LE16 fermentation liquid

KEGG ID	Library ID	CAS ID	Metabolite	Formula	Functions
C00156	–	–	P-salicylic acid	$C_7H_6O_3$	As a module in activating disease resistance[30]
–	HMDB0041054	158243-18-6	Ustiloxin D	$C_{23}H_{34}N_4O_8$	Antifungal activity[31]
–	HMDB0032549	50854-94-9	N-undecylbenzenesulfonic acid	$C_{17}H_{28}O_3S$	Antifungal activity[32]
C07235	HMDB0014753	72559-06-9	Rifabutin	$C_{46}H_{62}N_4O_{11}$	Broad-spectrum antibiotic (bacteria and fungi)[33]
–	–	–	Nanaomycin	$C_{16}H_{14}O_6$	Broad-spectrum antibiotic (bacteria and fungi)[34–35]
C12012	HMDB0014700	220620-09-7	Tigecycline	$C_{29}H_{39}N_5O_8$	Broad-spectrum antibiotic (bacteria and fungi)[36]
C07225	HMDB0015152	10118-90-8	Minocycline	$C_{23}H_{27}N_3O_7$	Broad-spectrum antibiotic (bacteria and fungi)[37]
C01468	HMDB0002048	108-39-4	M-cresol	C_7H_8O	Intermediate of antifungal pesticide[38]
C10438	HMDB0000567	621-82-9	Cinnamic acid	$C_9H_8O_2$	Antifungal activity[39]
C00557	HMDB0031407	120-92-3	Cyclopentanone	C_5H_8O	Antimicrobial[40]

–: unrecorded.

2.4　LE16 发酵液粗提物对灰葡萄孢基因表达的影响

RT-qPCR 验证结果表明，基因表达变化趋势与转录组测序结果基本一致(图 4)。对无(对照)和加粗提物处理的灰葡萄孢菌丝中的显著(P<0.001)差异表达基因(differently expressed genes，DEGs)进行 GO 分析表明，DEGs 与病菌 37 个生物学性状密切相关，这些生物学性状分别属于生物过程(biological process，BP)、细胞成分(cell component，CC)和分子功能(molecular function，MF)等(图 5)。在 BP 中，代谢过程(下调基因是上调基因的 4.81 倍)和细胞过程(下调基因是上调基因的 6.34 倍)的 DEGs 数量最多，分别占总 DEGs 的 19.51%和 21.28%；与之类似，

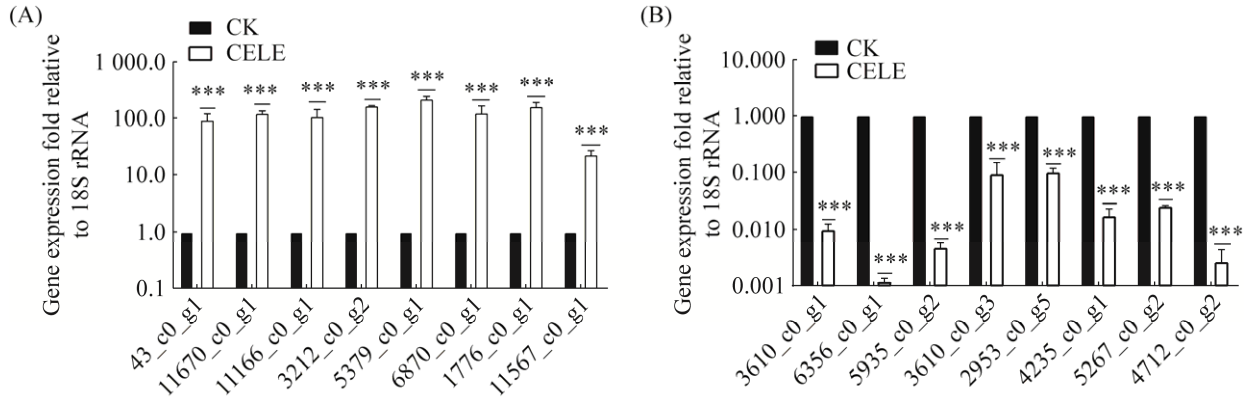

图 4　差异基因的 RT-qPCR 验证

Figure 4　RT-qPCR verification of differentially expressed genes. CK: control; CELE: crude extracts from LE16 fermentation liquid. ***: significant differences at $P<0.001$ (independent-sample t test).

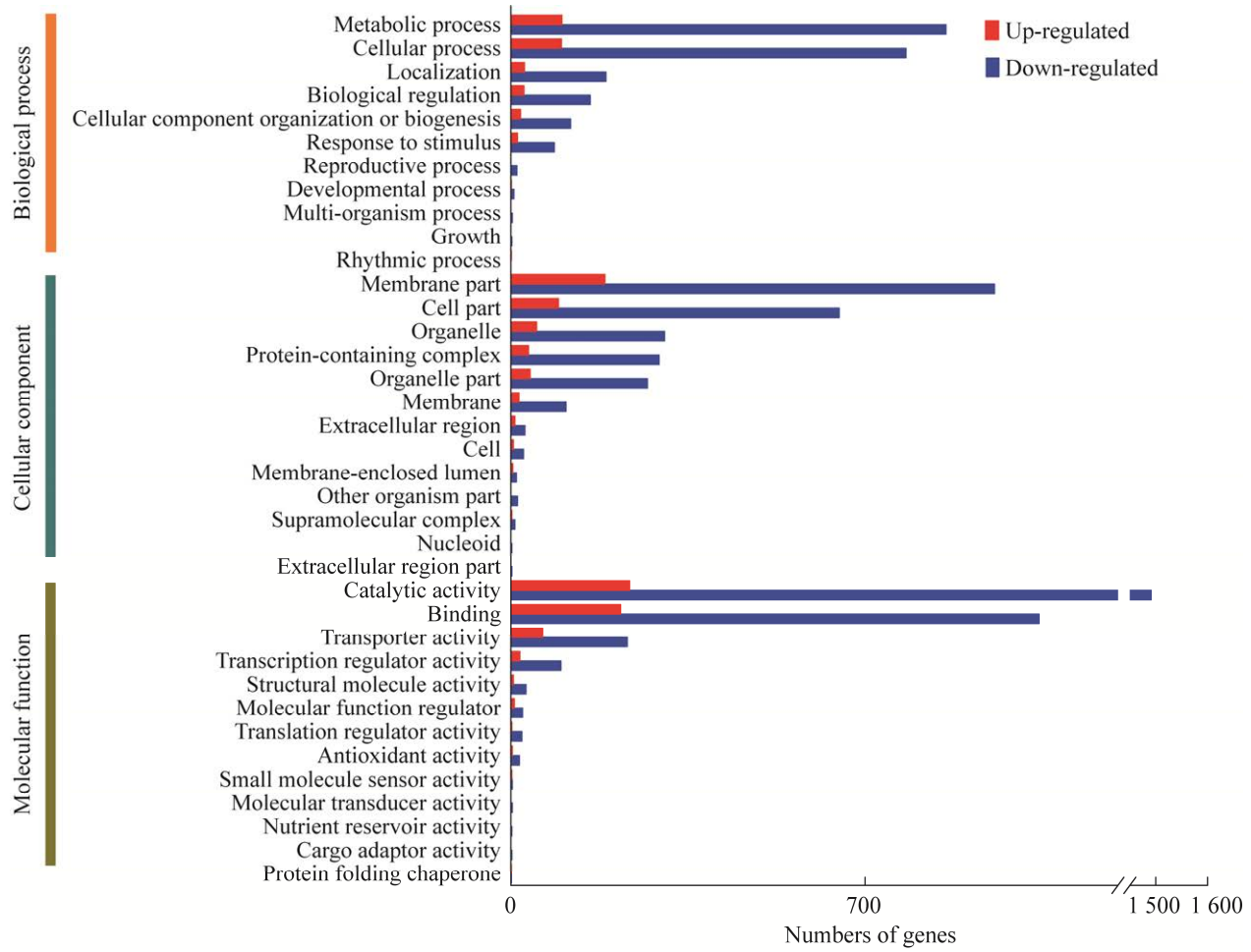

图 5　产酶溶杆菌 LE16 发酵液粗提物处理后灰葡萄孢差异表达基因注释的 GO 功能

Figure 5　The GO functions annotated by differentially expressed genes in *B. cinerea* with crude extracts from *L. enzymogenes* LE16 fermentation liquid.

CC中是膜组分(下调基因为上调基因的6.90倍)和细胞组分(下调基因为上调基因的5.14倍),分别占总DEGs的25.29%和16.45%。在MF中,催化活性(下调基因为上调基因的7.81倍)和蛋白结合(下调基因为上调基因的8.51倍)的DEGs居首,分别占总DEGs的38.22%和27.92%。此外,在BP中,与蛋白质翻译的tRNA氨基酰化、氨基酸的激活、囊泡介导转运、细胞对DNA损伤刺激的反应、细胞蛋白修饰、氨基酸代谢等相关的DEGs显著富集(图6)。在MF中,显著富集的DEGs涉及到氧化还原酶(结合或减少分子氧)、受损DNA结合、催化(作用于tRNA)活性等。

KEGG分析表明,DEGs显著富集的代谢通路包括氨酰生物合成、蛋白酶、错配修复、甘氨酸丝氨酸和苏氨酸代谢、脂肪酸生物合成、赖氨酸生物合成、组氨酸代谢等(图7)。

2.5 菌株 LE16 发酵液对温室番茄灰霉病的抑制作用

由表2可见,在CK处理中,番茄植株无灰霉病症状;在PI处理中,番茄灰霉病的病情指数为43.70,显著高于CF和LEFL的预防治疗处理(7.50–11.77)。防治效果为CF+PI (82.50%)>PI+CF (77.92%)>LEFL+PI和PI+LEFL (74.42%–72.54%,二者无显著差异)。

2.6 LE16 发酵液(LEFL)对番茄叶片抗氧化酶活性、膜透性和丙二醛含量的影响

由图6可见,PI处理的SOD活性显著高于CK;与PI相比,LEFL+PI和CF+PI显著提高了SOD和POD活性,PI+LEFL和PI+CF显著提高POD活性。在PI处理中,番茄叶片的丙二醛含量显著高于CK;在LEFL+PI、CF+PI、PI+LEFL和PI+CF处理中,番茄叶片的细胞膜透性和丙二醛含量显著低于PI (图8)。

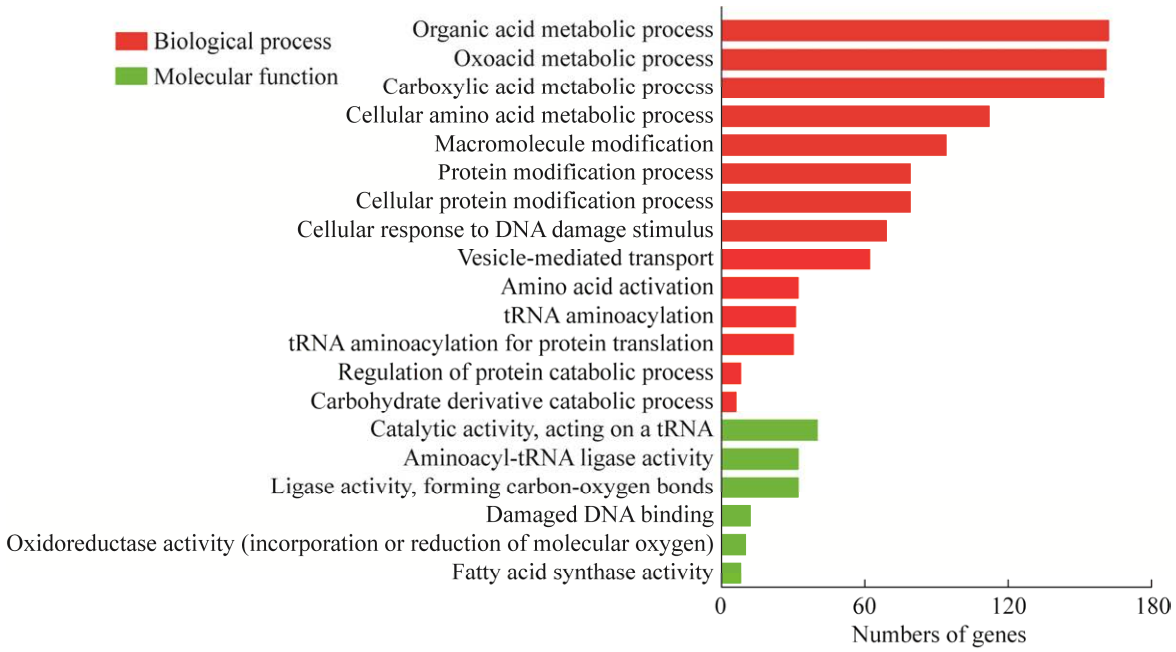

图6 产酶溶杆菌LE16发酵液粗提物处理后灰葡萄孢差异表达显著富集的GO功能

Figure 6 The GO functions significantly enriched by differentially expressed genes in *B. cinerea* with crude extracts from *L. enzymogenes* LE16 fermentation liquid.

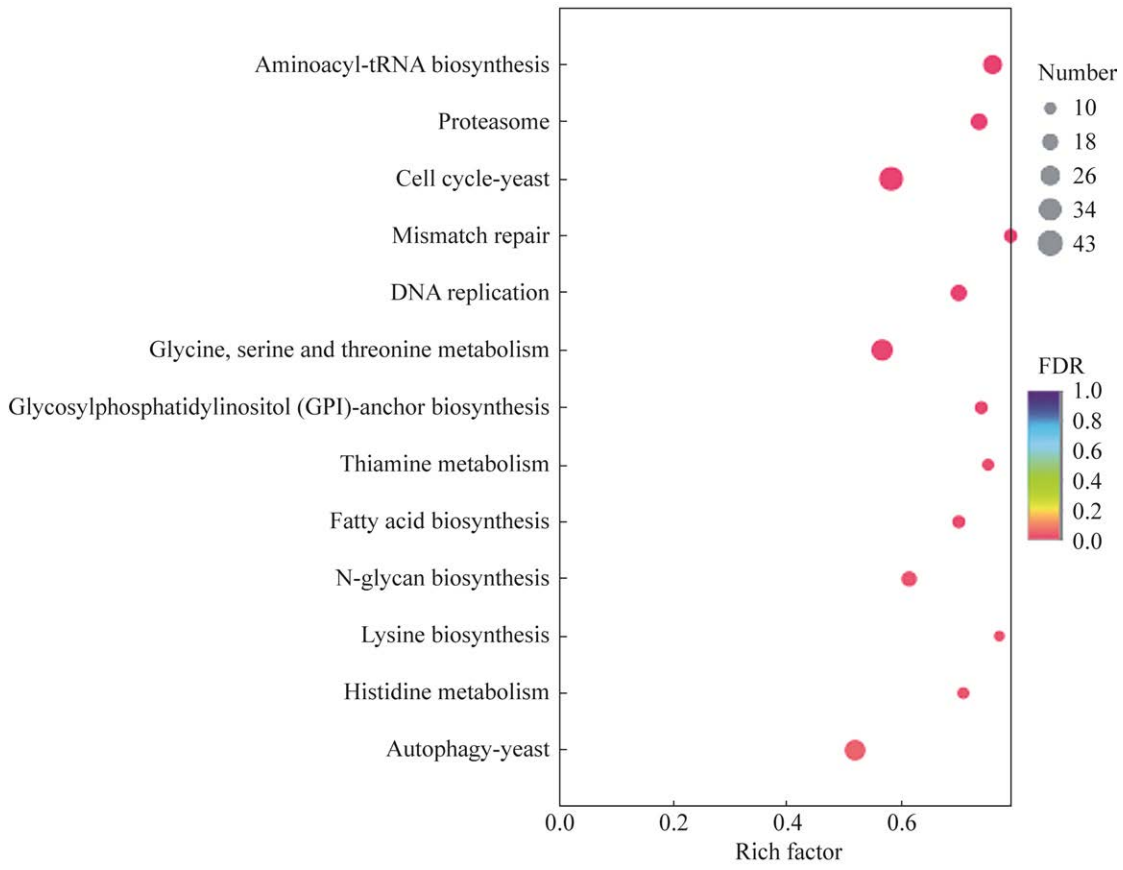

图 7　产酶溶杆菌 LE16 发酵液粗提物处理灰葡萄孢后差异表达基因显著富集的 KEGG 代谢通路

Figure 7　The KEGG pathways enriched significantly by differentially expressed genes in *B. cinerea* with crude extracts from *L. enzymogenes* LE16 fermentation liquid. The vertical axis shows the pathway names. The horizontal axis represents the rich factor, i.e. the ratio of the transcript number enriched in this pathway to the annotated transcript number. The higher rich factor indicates the greater enrichment degree. The size and color of the dots indicate the number of genes in this pathway and different *P*-values, respectively.

表 2　产酶溶杆菌 LE16 发酵液(LEFL)对温室番茄灰霉病的控制效果

Table 2　Suppression efficacies of *L. enzymogenes* LE16 fermentation liquid (LEFL) against tomato gray mold in greenhouse

Treatments	Disease index/%	Control efficacies/%
CK	0.00±0.00e	–
PI	43.70±6.22a	–
LEFL+PI	10.96±1.32b	74.42±4.79c
CF+PI	7.50±0.51d	82.50±2.79a
PI+LEFL	11.77±1.54b	72.54±5.27c
PI+CF	9.43±1.42c	77.92±5.19b

CK: control; PI: pathogen inoculation alone; LEFL+PI: the spray of LEFL prior to pathogen inoculation; CF+PI: the spray of chemical fungicide prior to pathogen inoculation; PI+LELF: the spray of LEFL after pathogen inoculation; PI+CF: the spray of chemical fungicide after pathogen inoculation. Values are means±standard deviation; different small letters indicate significant differences among different treatments at $P \leqslant 0.05$ (Duncan's multiple range test).

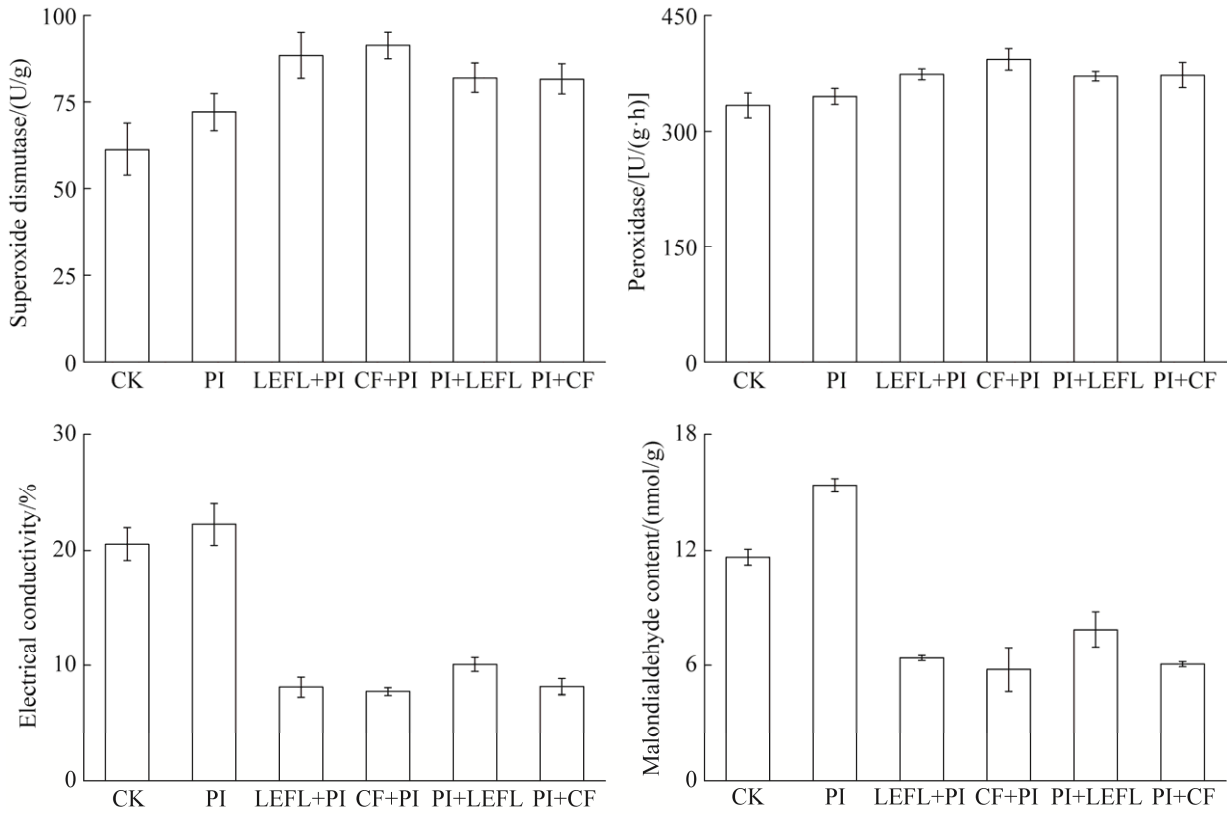

图 8　产酶溶杆菌 LE16 发酵液(LEFL)对番茄叶片抗氧化酶活性、细胞膜透性及丙二醛含量的影响

Figure 8　Effects of *L. enzymogenes* LE16 fermentation liquid (LEFL) on antioxidant enzyme activities, membrane permeability and malondialdehyde level in tomato leaves. CK: control; PI: pathogen inoculation alone; LEFL+PI: the use of LEFL prior to pathogen inoculation; CF+PI: the use of chemical fungicide prior to pathogen inoculation; PI+LEFL: the use of LEFL after pathogen inoculation; PI+CF: the use of chemical fungicide after pathogen inoculation. Values are means±standard deviation; different lowercase letters indicate significant differences at $P \leqslant 0.05$ (Duncan's multiple range test).

3　讨论与结论

3.1　菌株 LE16 水解酶和抗菌物质的产生

LE16 能分泌磷酸酶、蛋白酶、纤维素酶、β-1,3-葡聚糖酶和溶菌酶。若这些酶作用于自身成分，可能引起自溶作用[41]。此外，磷酸酶和蛋白酶除活化土壤有机氮磷，改善植物营养外，还能使生防菌在水解利用氮磷方面比病原菌更具竞争力[42−43]。植物的病原真菌(如疫酶)的细胞壁含纤维素和 β-1,3-葡聚糖，故 LE16 分泌纤维素酶和 β-1,3-葡聚糖酶可水解破坏病原菌的细胞壁[14,42]。此外，LE16 能够产生铁载体，属一类分子量为 200–2 000 Da 的螯合物，可螯合铁离子，有利于生产者吸收铁，降低环境中的有效铁，造成病原菌缺乏铁营养素[44]。因此，产生水解酶和铁载体可视为 LE16 拮抗植物病原真菌的机制之一。

LE16 发酵液粗提物显著抑制灰葡萄孢的生长繁殖。LC-MS 分析发现，LE16 发酵液含毒菌素 D、利福布汀、纳奈霉素、替加环素、米诺环素、间甲酚、肉桂酸、环戊酮和 N-十一烷基苯磺酸等 9 种抗真菌物质。目前，人们已

揭示了这些抗真菌物质的作用机制。例如，毒菌素 A–F 抑制 α,β-微管蛋白二聚体进入微管，从而抑制真核细胞的有丝分裂[45]，已在医药和农业上用于抗癌、抗真菌或驱虫等[31]。利福布汀含有螺哌嗪基，是利福霉素的一种衍生物，具广谱抗菌活性，其作用机制类似利福平，即通过与微生物 DNA 依赖的 RNA 多聚酶 β 亚基稳定结合抑制其活性，从而阻碍病原菌的 RNA 合成[33]。纳奈霉素 A–E 在还原过程中会产生超氧阴离子(O^{2-})，对病原菌造成多方面的危害[34–35]。替加环素内含甘氨酰环肽，属于第 3 代四环素类抗生素，其抗菌机理是抑制病菌生物膜形成[36]。此外，有些生防菌还能分泌信号分子，被植物细胞膜上的特定受体识别，诱发植物的多重免疫反应，最终产生系统抗病性[46]。其中，水杨酸是介导植物由局部免疫抗病反应到产生系统抗性的重要信号分子之一，外源水杨酸及其衍生物也能使许多植物对真菌、细菌和病毒等多种病原微生物产生系统抗性[30]。由此可见，LE16 能分泌多种抗真菌物质，它们有各自的作用靶点，多种抗菌物质同时存在，可能有益于增强抑菌效果，但究竟是哪种或哪几种抗菌物质起主导作用尚需进一步研究。

3.2 LE16 发酵液粗提物对灰葡萄孢生化参数和基因表达的影响

LE16 发酵液粗提物显著影响灰葡萄孢基因的表达，在灰葡萄孢下调表达的基因中，与抗氧化、翻译、转录、转运、催化活性等相关基因显著富集，意味着 LE16 发酵液粗提物从总体上对病菌生命活动产生负面影响，并抑制抗氧化酶相关基因的表达而降低灰葡萄孢消除活性氧的能力。KEGG 进一步分析发现，经 LE16 发酵液粗提物处理灰葡萄孢后，氨酰生物合成、错配修复、DNA 复制、甘氨酸、丝氨酸和苏氨酸代谢、N-多糖生物合成等代谢途径的 DEGs 显著富集，表明 LE16 合成分泌的代谢产物显著影响病菌蛋白质合成和 DNA 复制等。甘氨酸是谷胱甘肽的组成氨基酸，谷胱甘肽具有抗氧化和综合解毒作用[47]。在细胞膜上，一些信号蛋白含有丝氨酸[48]，其代谢途径发生变化可能影响信号转导及下游生理生化反应；苏氨酸结构中的羟基可与寡糖结合，保护细胞膜免受损伤[49]。因此，LE16 发酵液粗提物对甘氨酸、丝氨酸、苏氨酸代谢的影响可能干扰病原菌体内的多个生理学和生物化学过程。

3.3 LE16 发酵液对番茄灰霉病的防治效果

LE16 发酵液对番茄灰霉病的抑制作用(72.54%–74.42%)略低于化学农药(77.92%–82.50%)。就病原菌而言，LE16 发酵液中含有与抗病性相关的多种酶类、铁载体和抗真菌物质，抑制灰霉病菌生长繁殖。就植物而言，LE16 发酵液显著提高了番茄叶片 SOD 和 POD 活性，有益于消除番茄感染病菌后产生的活性氧，降低对膜脂和细胞器的伤害[50–51]，致使病害减轻，番茄叶片膜透性和丙二醛含量下降。植物抗氧化酶活性增强是植物产生诱导系统抗性(induced system resistance，ISR)或系统获得性抗性(systemic acquired resistance，SAR)的表现之一[51–52]。ISR 主要由有益生防促生菌激活，SAR 由病原菌诱导，需要水杨酸和病程相关蛋白等信号分子参与[53]。LE16 发酵液中含有水杨酸，因此有助于番茄植株产生 SAR。

综上所述，LE16 能合成分泌铁载体、多种抗病性相关的酶类和抗真菌物质，其发酵液显著抑制灰霉病菌的繁殖生长、影响灰霉病菌基因的表达，这些差异表达基因涉及多个生物学性状和代谢通路，诱导番茄植株产生抗病性，抑制温室番茄灰霉病，具有潜在的应用价值。

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